"Studies on the breakdown of 2,4-dichlorophenoxyacetic acid and some related compounds by soil micro-organisms".

By Norman Brownbridge.

Thesis submitted for Ph.D. degree, September, 1956.

The results presented in this thesis were obtained while I was acting as Research Assistant to Professor L.J.Audus. My thanks are due to him for permission to use them in this way and also for his help and advice at all times.

My thanks are also due to the Agricultural Research Council who financed the research from which the results were obtained.

Monsanto Chemicals Limited kindly supplied some of the phenols used, as well as a number, of which time did not permit investigation.

INDEX.

Abstract.

Modifications of the perfusion and cress-assay techniques of Audus were used to study breakdown in soil of 2,4-D and some related compounds.

Attempts to produce active bacterial (pure) cultures from the enriched soils proved abortive. Mixed, crude cultures were partially successful though never energetic. A wide range of stimulants failed to induce pure culture activity.

Enrichment was transferrable to fresh soil by treating it with perfusate from an active perfuser. The activating principle was thermolabile, probably consisting of adapted bacteria. The technique was used to quickly produce numbers of similar enriched perfusers without the neccessity of lengthy adaptation processes.

Compounds labile in soil followed the same general course, a characteristic lag phase being followed by a period of rapid breakdown. Further added substrate was usually decomposed with no further lag. Some control of the lag was obtainable by pre-treatment of the soil, or perfusion in mixture, with related compounds. Mixtures of certain phenoxyacetic acids appeared to be synergistic in their inhibition of root growth.

2,4-D adaptation proved very stable, persisting through prolonged perfusion with water, or storage for over a year, of enriched soil.

The optical isomers of the chlorophenoxypropionic acids appear to differ in resistance to attack as well as having different physiological activities.

Relative Toxicity to cress and lability in soil appear to be controlled by the same molecular features and a close correlation exists between the two functions. Attachment to substrate (enzyme?) in each case is probably through the α-H and carboxyl groups of the side-chain and the 3- or 5-nuclear positions.

The effects of molecular substitution on stability and physiological activity are discussed as also are the possibilities of phenol formation and / or ring fission during breakdown.

A possible relationship between the breakdown products and biotin metabolism is also indicated.

Introduction.

Although phenoxyacetic acid, and a number of its derivatives, has been known for many years, it is only fairly recently that the growth regulating properties of certain of them have found practical application.(For historical surveys see, 1, 10, 125, 139.).

The impact of World War II, and the need for more efficient food production, stimulated research into better methods of weed control. The introduction of substituted phenoxyacetic acids, and their derivatives, as selective herbicides was a result of investigations by several workers in Britain and the United States. Perhaps most of the credit should go to Slade, Templeman and Sexton (107) and Nutman, Thornton and Quastel (92) who demonstrated the practical value of these compounds.

The use of selective herbicides has necessitated a study of many interacting factors such as method and season of application, climate, soil structure, stage of plant development and the range of susceptibility covered by different species and varieties. Much effort has been expended in an endeavour to correlate herbicidal and other physiological activity with molecular structure of active substances. (59, 61, 116, 117, 121.). Many thousands of compounds have, to this end, been synthesised and tested. Crafts and Harvey (24) have discussed this relationship from the herbicidal point of view while Veldstra (127) has produced an admirable critical survey of the field generally.

In 1935 Hitchcock and Zimmerman (51) applied various growth regulating substances to soil and observed their uptake by, and effects on, plants growing in the soil. By 1940, Slade et al (107) claim to have demonstrated the control of charlock in cornfields by applying α-naphthyl-acetic acid, as a spray, directly to the soil. They later found that certain substituted phenoxyacetic acids were cheaper and more efficient. Nutman et al (92) confirmed these findings and showed that the soil was unlikely to be permanently affected by this kind of treatment. Established weeds were affected and even the seeds of susceptible species were killed, in the soil, before the seedlings could emerge. It is largely from these beginnings that the pre-emergence technique of weed control has developed.

When selective herbicides are used for such purposes as pasture improvement or scrub clearance, persistence in the soil is of little importance. On the other hand, for pre-emergence treatment to be of value in arable farming, horticulture and similar practice, the degree of persistence is a critical factor. This is especially true when it is desired to make a subsequent planting of a susceptible crop in the treated soil. To be of practical value, the herbicide must show some persistence in the soil, but this should be no greater than necessary. The ability to predict and control persistence would be a great practical asset. Ideally, the herbicide should persist in the surface layers of the soil just long enough to kill the weeds and weed seeds present, yet should

disappear before the crop seeds are sown, or before they germinate if they have been sown previously at some depth. When applied in the presence of seedling, or mature, crops, of resistant species, the herbicide should disappear before leaching can carry it down to lower levels in the soil where toxic concentrations might possibly develope around the crop roots.

The accidental or deliberate contamination of soil by selective herbicides has, thus, created another group of problems, the "interaction of herbicide and soil", of which that of persistence is probably the most important. Much attention has been payed to these problems, particularly in America, and something, at least, is known about most of the factors involved. The disappearance of 2,4-D, and other selective herbicides, from soil, under both field and laboratory conditions, has been studied. Many workers (2, 7, 9, 16, 23, 27, 28, 43, 44, 47, 48, 52, 56, 57, 59, 62, 71, 72, 77, 81, 86, 87a, 89, 90, 92, 97, 123, 126, 131,) have determined the persistence of 2,4-D in soil under a variety of conditions. Periods of from 10 days to 18 months have been quoted, depending on many edaphic and climatic conditions and on the amount of herbicide applied. Much of the earlier work lacks precision, as the usual test for disappearance of toxicity was to make successional sowings of susceptible crops, in the soil, till normal germination was observed. In later work, greater accuracy has been achieved by assaying, biologically, soil extracts, leachates or perfusates (7, 9,

44, 89, 90,). The operation of individual factors in herbicide disappearance has been studied intensively. Some workers have reported that leaching does not occur readily (23, 44, 86, 92,) Others have shown 2,4-D and its salts to be more or less readily leached from the surface to deeper layers in the soil (44, 48, 77,), and that the amount of leaching is proportional to the amount of rainfall and concentration of herbicide used (44, \, 92,). The effect of soil structure and constitution on leaching and physical inactivation has been studied (2, 16, 23, 44, 47, 86, 92,) and may be compared with detoxication experiments using ion-exchange resins and activated carbon (73, 131, 132,).

Though leaching may be of importance under field conditions, it is obviously not the only detoxication mechanism for it cannot account for the loss of activity in stored soil mixtures (16, 23, 27, 28, 47, 48, 59, 62, 72, 81, 88, 89, 90, 92,). Physical adsorbtion and chemical degradation have also been proposed as ways in which 2,4-D might disappear. Adsorbtion may be deduced from the results of the leaching and other experiments mentioned above. Audus (9,) measured the adsorbtion of 2,4-D by one soil. He found it to be small and to take place rapidly. It cannot, therefore, account for the normal, prolonged detoxication process in soil. Akamine (2,) could find no correlation between persistence and soil adsorbtive capacity, but persistence and toxicity to test plants has been shown to vary with soil type (11, 17, 92,). Chemical degradation also appears to be of little importance, for 2,4-D has been shown

to persist for upwards of 18 months in autoclaved soils (16, 28,). Adverse effects of autoclaving, eg. on the soil colloids, cannot be ruled out in this arguement.

The factors outlined above are not enough to account for the normal disappearance of 2,4-D and related herbicides from soil and, as these compounds are not appreciably volatile, we are left with the alternative that the disappearance is due to micro-biological degradation. Much circumstantial evidence has been accumulated in favour of this theory. A number of investigators (2, 16, 22, 28, 42, 47, 48, 57, 59, 62, 71, 72, 81, 131,) have observed the accelerating effect of high soil moisture and temperature and/or the retardation of cold and dry conditions. 2,4-D has been shown to persist in autoclaved soils (16, 28, 47, 48,), and to have an increased rate of breakdown in soil normally high in organic matter or to which manure has been added (16, 44, 47, 48, 57, 62, 81, 92,). Though all these factors affecting 2,4-D breakdown probably do so by controlling the metabolism of micro-organisms, they could produce similar effects on a purely chemical degradation.

It is generally assumed that soil bacteria are responsible for 2,4-D breakdown and it might therefore be expected that the rate of breakdown would be greater in neutral or slightly alkaline soils where conditions would be more suitable for bacterial proliferation. Little correlation has been found between soil pH and optimum breakdown rate. It has been said to disappear faster at higher pH values (2,), lower pH values (23, 44, 62,) and to be unaffected by pH (59,).

In this connection, reference may be made to the work of Fromageot and Mayer (36,) who showed that,under acid soil conditions, a non-biological oxidation of organic compounds could result from the simultaneous adsorbtion of these compounds with molecular oxygen onto the soil colloid surfaces. Under neutral, and alkaline, soil conditions, biological oxidation was shown to prevail. The lack of agreement on soil pH for optimum 2,4-D breakdown may be due to variation in the relative importance of biological and non-biological oxidation under the test conditions.

As circumstantial evidence favours the theory of biological oxidation of 2,4-D attempts have been made to isolate effective micro-organisms,(104,). Martin (77,) inoculated sterile media, containing 2,4-D as the sole carbon source, with a) fresh soil, and b) soil whichh had been previously incubated with 2,4-D. He measured the evolution of carbon dioxide and showed that it was higher from the previously treated suspension and sufficient in amount to indicate breakdown of the 2,4-D by the soil population. Using the soil perfusion technique, Audus (7,) produced further indirect evidence of bacterial breakdown and, in subsequent papers (8, 9,) claimed the isolation of an effective organism. It belonged to the very common group of soil bacteria "Bacterium globiforme" (69,), the type species of which was first described by Conn (20,). It was capable of utilising 2,4-D as sole carbon source on solid media. 2,4-D solution, perfusing through fresh soil,showed a rapid breakdown when inoculated with a suspension of the bacteria.

The work of Newman and Thomas (89,) and Newman and Norman (88,), who used soil incubation methods, largely confirmed the findings of Audus. They, following Martin (77,), showed that mixed cultures from treated soils were more active than those from untreated soils. Not one of the pure cultures,which they obtained, proved to be very active and it was suggested that certain micro-nutrients, or a more readily available carbon source, might be required if pure cultures were to break down 2,4-D efficiently. Anderson and Baker (3,) reported that they could not demonstrate the breakdown of 2,4-D by soil micro-organisms in culture media. Akamine (2,) showed that the rate of 2,4-D disappearance was positively correlated with the aerobic bacterial count in Hawiian soils.
Jensen and Petersen (56, 57,) isolated two species of bacteria, capable of decomposing 2,4-D, from repeatedly treated soil. They identified one as Flavobacterium aquatile and the other closely resembled the Bacterium globiforme of Audus (8,).

From the work of Audus (9,) and Newman and Thomas (89,), it is clear that when 2,4-D is applied to fresh soil there is a rapid but small drop in activity due, presumeably, to physical adsorbtion. This is followed by a stationary, or lag, phase of fairly short duration (1 to 6 weeks) during which the concentration does not change significantly. A third phase then begins during which the 2,4-D concentration rapidly falls to zero. These observations are in agreement with the hypothesis that in some way the 2,4-D induces, or permits, the selective proliferation of a bacterial population adapted to

the breakdown of 2,4-D and that the detoxication curve parallels the proliferation curve of the adapted organisms. In this way a stable, adapted population is thought to arise. Laboratory (7, 9, 56, 57, 89, 90, 91,) and field (90, 91,) experiments tend to support this view for it is found that subsequent 2,4-D treatments are never as persistent as the first, though there is a tendency to achieve a maximum detoxication rate which may be regarded as a sign of bacterial saturation of the soil.

Newman, Thomas and Walker (90,) showed that with soils taken from different depths of a profile, the duration of the lag, or adaptation, phase varied directly with the depth (presumeably,inversely with the corresponding bacterial count), being 14 days in the 0 to 6 ins. layer and 42 days in the 18-21 ins. sample. There was much less variation on retreatment of the same samples, indicating saturation of the soil with adapted bacteria during the first treatment. Brown and Mitchell (16,) observed that a given amount of 2,4-D disappeared more rapidly when mixed with the soil, rather than just applied to the surface. This also is in accord with the above ideas, for mixing the 2,4-D into a larger volume of soil would increase the number of contacted bacteria from which an adapted population could develope. The accelerating action of cropping and good soil management (23, 62,) on the rate of 2,4-D disappearance can also be attributed to effects on the number and distribution of bacteria throughout a larger mass of soil. Improved aeration may also play a part in the increase.

It would seem that in properly managed, normal, agricultural soils the disappearance of 2,4-D can be attributed, almost exclusively, to the activities of a bacterial population,possessing the required enzyme system, which proliferates from the normal soil population in response to selective stimulation by the 2,4-D itself. It cannot be said as yet whether this 2,4-D adapted population arises from 2,4-D induced mutant cells, or whether it is a selective proliferation of a minority of capable cells already existing among the normal soil population.

That the adapted population is stable, once it has arisen, was shown by Newman et al (90,) who found soil to possess 2,4-D detoxicating activity one year after previous treatment. There is a degree of specificity in the adaptation reaction as was shown by Newman and Thomas (89,) who studied the effect, on 2,4-D adaptation, of pretreating the soil with structurally related compounds. Audus (9,) showed that some specificity existed even towards the closely related compounds 2-methyl,4-chlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid. Jensen and Petersen (56,) similarly found that their organism No.2 would break down both 2,4-D and MCPA, but their organism No.1 (Flavobacterium aquatile) would only break down 2,4-D.

The action of micro-organisms on 2,4-D has received considerable attention, but the effects of 2,4-D, and related compounds, on micro-organisms has probably received more (3, 15, 19, 22, 25, 29, 37a, 57, 68, 74, 77, 79, 87, 94, 96, 111, 117, 118, 138,). Many of the results

are, however, contradictory. This may be due in part to the variety of methods and media used in the studies. It is also probable that in some cases, commercial 2,4-D was used which may contain as much as 30% impurities, consisting of various phenols and phenoxyacetic acids, which may be assumed to modify its activity considerably. Hansen (45,) has shown that similar impurities in commercial MCPA have marked effects on its herbicidal activity. It seems generally agreed that 2,4-D has no marked bactericidal or bacteriostatic properties. Effects, even on sensitive species, only become apparent at concentrations above 100ppm.(15, 19, 57a, 58, 68, 74, 76, 96, 111, 118,). On the whole, fungi are even less sensitive than bacteria (15, 25, 74, 118,) and it has been reported that 4% 2,4-D is required to inhibit Rhizopus nigricans (25,) At this concentration many normal metabolites would probably prove toxic.

It would appear that the undissociated molecule is more toxic to micro-organisms than is the free ion, for greater toxicity is manifest in media of lower pH values (23, 29, 77, 87,). The theory is also supported by Newman's (87) observation that the 2,4-D esters are equally toxic at all pH conditions. An explanation of these observations, and of the similar relationship of herbicidal activity to pH, has been proposed by Crafts (22a, 23,).

Among the more sensitive bacteria are some of those concerned in the nitrogen cycle though, again, opinions differ concerning the relative sensitivity of various genera (19, 37a, 57a, 58, 68, 87, 88, 96, 111, 117,).

Cellulose decomposing organisms such as Cellvibrio, Cytophaga and Sporocytophaga (57, 117,) also appear to be sensitive, though it is interesting to note that Jensen and Sörensen (57a,) found some concentrations of 2,4-D to stimulate carbon dioxide output from soil into which lucerne meal had been incorporated.

Worth and McCabe (138,) found that anaerobes tended to be inhibited, especially at higher concentrations, whereas aerobes tended to be unaffected, or even stimulated, Stapp and Freter (117,) found all the spore formers they tested to be sensitive, whether aerobic or anaerobic. The vast majority of soil micro-organisms, fermentative and non-specialised, are apparently quite insensitive to 2,4-D and it requires concentrations as high as 0.5% to reduce carbon dioxide output significantly (87,).

It would seem that the fairly low concentrations of 2,4-D normally employed in weed control practice have no marked effect on a normal soil micro-population, or on pathogens (68,). Any effect which may be produced is ephemeral (3, 76, 111, 117,) and Marcelli (76,) even showed the initial drop in viable count (due to arrested multiplication) to be temporary and surpassed the starting level when division recommenced.

A possible first step in the bacterial degradation of 2,4-D, and related herbicides, would be for the ether linkage to be severed. An aliphatic acid would probably form from the side chain and the corresponding halogenated phenol from the nucleus. The fate of the herbicides would then

resolve itself into a study of the degradation of halogenated phenols. Numerous workers (14, 30, 31, 40, 60, 105, 115, 141,) have, in the past, studied the breakdown of aromatic compounds, including phenol and cresols, by soil and other bacteria. Following on this preliminary work, Evans et al (30, 31,) and Stanier et al (108, 109, 114, 115, 116,) worked out the main pathway of phenol degradation and others have filled in the details. The much more difficult problem of halogenated phenol breakdown seems to have been completely neglected. The bactericidal properties of some of these compounds have been studied (22, 79, 94,) and they appear to be markedly active compounds. Their specific activity depends on a number of factors such as, (a) position of substituents, (b) number and character of substituents, (c) concentration of undissociated phenol which in turn depends on the pH of the medium.

Apart from their possible role as intermediates in the breakdown of the corresponding phenoxyacetic acids, the phenols are also of interest because of the extent (even as much as 20%) to which they occur in commercial grade herbicides, It is not inconceivable that phenol, added to the soil in this way, could interfere with the metabolism of the bacteria responsible for the breakdown of the phenoxyacetic acids. This may be partly responsible for the contradictory results obtained in some field trials.

Evidence has been produced to show that pentachlorphenol (71, 72,) and 2-chlorphenol (129,) are subject to bacterial decomposition in soil and that 4-chlor-

phenol and 2,4-dichlorophenol are more resistant than 2-chlorphenol.

It will be seen from the foregoing that much is known of the conditions under which 2,4-D will, or will not, disappear from treated soil. In addition, bacteria which are believed to be largely responsible for the disappearance, have been isolated in pure and mixed cultures from treated soils(8, 9, 56, 89,). Some progress has been made in the related field of study, the fate of 2,4-D in plants, where the radio-isotope techniques have been of value (32, 32a, 53, 54, 55, 133, 134, 135, 136,), but no attempt has been made to study in detail the degradation of 2,4-D, and related compounds by soil micro-organisms.

The primary aim of the present investigation was a study of the kinetics of breakdown of 2,4-D and related compounds, in a closed system containing fresh soil, under fairly constant conditions of temperature, moisture and aeration. The soil perfusion technique (6, 7, 9, 64, 65, 66, 100,) was adopted as being the most convenient and suitable for the purpose. It was hoped that from the enriched soil, produced by perfusion, pure cultures of adapted bacteria would be obtainable. Using these, in entirely synthetic liquid media, a detailed study of the breakdown mechanism of these compounds was to be made. Where possible, intermediate metabolites were to be isolated. Though some study of the organisms, believed to be responsible for the breakdown in soil, was made, breakdown could not be achieved using cultures in purely synthetic media. This part of the research proved

abortive.

As well as following the disappearance of single compounds, the fate of mixtures of compounds was also studied, especially the interaction of the components and the adaptative response of the soil population towards each component.

If a stable, enriched soil can be regarded as a single metabolising unit, in the same sense as a pure culture, then it should be permissable to apply the simultaneous adaptation technique (108,) to such a soil, in order to determine whether common metabolic pathways exist in the degradation of related compounds. For this reason, the effect of enriching soil to one compound and its subsequent behaviour towards a second compound was studied. From the results of these varied perfusions it was hoped that information would be obtained regarding a possible relationship between molecular structure and resistance to breakdown and, also, an indication of the primary point of attack on the molecule. From the practical viewpoint, answers to two other problems were sought, (a) how to control the persistence of herbicides in soil in order to achieve maximum safe effect with minimum loss of cropping time; this by control of the lag phase, and (b) whether it would be possible to operate a rotational system of herbicidal treatment, using various substituted phenoxyacetic acids, in soils already active against 2,4-D and MCPA, and in which it would probably be impossible to maintain an effective concentration of either of these two compounds.

Audus (7, 9,) and Newman et al (88, 89, 90, 91,) made some study of the kinetics of breakdown of 2,4-D, MCPA and 2,4,5-T but, so far as is known, no similar work has been carried out on other substituted phenoxyacetic acids. These authors also gave the results of some pretreatment experiments, though they did not cover the range of compounds included in the present research.

The attempt to discover a relationship between molecular structure and breakdown by bacteria, in the present context, is also virtually a new problem. Those experiments associated with attempts to control the persistence of herbicides in soil are also original.

Materials.

The basic materials for this research were soil and herbicidal or related compounds. It was decided to restrict the study to one soil source and to examine its reactions towards a larger number of herbicidal and related compounds, for it was felt that such a course would be more likely to provide some answers to the important questions, (a) how are these selective herbicides detoxicated by soil bacteria, and (b) is there a relationship between molecular structure and resistance, or lability, to bacterial attack. Persistence of acquired detoxicating activity could be simultaneously determined.

1. Soil.

For the sake of convenience and the absence of possible interference, the soil chosen was from a neglected garden in N.W.London (Sussex Lodge, Sussex Place, London, N.W.1) For a number of years the soil had received little more attention than an annual digging over, though some attempt had been made to grow potatoes in it. Large samples (at least three pounds) of the soil were collected, as required, from the top six inch layer. The soil was fairly light, with a low organic and low clay content but containing a high proportion of sand (fine). In the fresh state it contained a fair proportion of small stones which were removed during the preparation of the soil. The final product was very nearly neutral in reaction. The fresh soil was spread in thin layers on sheets of paper and air dried. Drying was facilitated by

occasionally turning over the layers and by blowing air over it using an electric fan. When thoroughly air-dry, the larger aggregates were broken down and the whole carefully sieved. That fraction passing a 4mm., but retained on a 2mm., sieve was found to function best in the type of perfuser used. This fraction constituted about 20% of the whole dried soil. Batches of about 500 grams were prepared, thoroughly mixed and stored in screw-capped glass bottles.

2. Chemicals.

These consisted largely of chlorinated phenoxy-acetic acids and chlorinated phenols, together with a few related compounds. Where possible they were obtained from normal trade sources but, in some instances, recourse had to be made to synthesis. The following is a complete list of the compounds studied, together with the abreviations used, throughout the rest of the text, to denote them, along with an indication of the source of each compound.

Full name.	Abreviation.	Source.
(1). Phenoxyacetic acid.	PAA.	PAL.
(2). 2-chlorophenoxyacetic acid.	2-CPA.	PAL.
(3). 3-chlorophenoxyacetic acid.	3-CPA.	PAL.
(4). 4-chlorophenoxyacetic acid.	4-CPA.	PAL.
(5). 2,4-dichlorophenoxyacetic acid.	2,4-D.	PAL.
(6). 2,5-dichlorophenoxyacetic acid.	2,5-D.	PAL.
(7). 3,4-dichlorophenoxyacetic acid.	3,4-D.	SYN.1.
(8). 2,4,5-trichlorophenoxyacetic acid.	2,4,5-T.	PAL.

Full name.	Abreviation.	Source.
(9). 4-chloro,2-methylphenoxyacetic acid.	MCPA.	ICI.
(10). 2,4-dimethylphenoxyacetic acid.	2,4-DM.	PAL.
(11). 4-iodophenoxyacetic acid.	4-IPA.	SYN.2.
(12). α-(4-chlorophenoxy)propionic acid.	α-4-CPP.	PAL.
(13). α-(2,4-dichlorophenoxy)propionic acid.	α-2,4-DCPP.	PAL.
(14). 2-chlorphenol.	2-CP.	HW.
(15). 4-chlorphenol.	4-CP.	HW.
(16). 2,4-dichlorphenol.	2,4-DCP.	PAL.PUR.

Substances labeled PAL were obtained from Pal Chemicals, a subsidiary company of Universal Crop Protection Ltd., London. They were claimed to be of a high degree of purity and to have sharp melting points. The 2,4-DCP labeled PAL.PUR. had been supplied originally by Pal Chemicals as pure. The crystals had become contaminated by a brownish liquid, probably as a result of spontaneous oxidation. It was purified by steam distillation, ether extraction of the distillate and finally a straight distillation of the phenol. The fraction distilling between 208 and 210°C was collected. It had a fairly sharp melting point of 44°C (uncorrected).

Compounds designated HW. were ordinary laboratory grade chemicals obtained from Hopkins and Williams, Chadwell Heath, Essex. No attempt was made to purify them further. Though normally better than 95% pure, the impurities were likely to be related mono- and dichlorphenols. In view of the observed effects of minority compounds in mixtures, the results obtained with these two compounds are not above suspicion.

ICI. The MCPA had been supplied originally by I.C.I. (Dyestuffs Division). It was slightly coloured but seemed to be of a high degree of purity.

SYN.1. and SYN.2. These two compounds were not available commercially. They were synthesised from technical grade phenols supplied by Monsanto Chemicals.

Synthesis 1.

3,4-D was prepared according to the method of Synerholm and Zimmerman (121,). To the sodium dichlorophenoxide, produced by dissolving 0.7gm. of sodium in 20ml. of absolute alcohol and then adding 5gm. of 3,4-DCP, was added 5.2gm. of ethyl bromoacetate. Sodium bromide began to precipitate at once and the mixture was maintained at 98°C for 1 hr. 20mls. of 10% sodium hydroxide solution was added and the mixture refluxed for 1 hr. Most of the colour was removed by boiling with charcoal and the liquor filtered hot. The acidified filtrate was stored overnight at 0°C. The crude acid was filtered off and air dried. Yield was 5gm. with a melting point of 127-133°C. It was dissolved in the minimum quantity (70ml.) of boiling benzene and poured off from a slight insoluble residue. 70ml. of petroleum ether (50-60°C) was added and the mixture again refrigerated overnight. The crystalline precipitate was washed with cold petroleum ether and dried. It had a melting point of about 135°C. The acid was quite soluble in boiling water but had a low solubility at temperatures only a few degrees lower. An even purer product was obtained, by repeated crystallisation from boiling water, as a white powder melting at 137-138°C (uncorrected).

Synerholm and Zimmerman (121,) give the melting point as 141°C.

Synthesis 2.

4-IPA was prepared by the method outlined by Newman et al (87a ,). To a solution of 10gm. of crude 4-iodophenol in 50ml. of methylated spirit was added 25gm. of potassium carbonate in 35ml. of water, followed by 7.5gm. of ethyl bromoacetate. The mixture was stood at room temperature overnight. Sufficient water was added to just dissolve the potassium bromide formed and the excess potassium carbonate. 3gms. of crude ethyl 4-iodophenoxyacetate was filtered off and dried. 2gms. of this crude product was refluxed for several hours with 100mls. of ethyl alcohol, 20mls. of water and 5gms. of potassium hydroxide. Most of the alcohol was distilled off, the residue taken up in 150ml. of hot distilled water, cooled and filtered. The precipitate, formed on lowering the pH with concentrated hydrochloric acid, was filtered off, dissolved in 100ml. of warm dilute ammonia solution, cooled and again filtered. The acid was again reprecipitated with HCl, filtered off, well washed with cold distilled water and air dried. 1.2gms. of pure 4-iodophenoxyacetic acid was obtained with a melting point of 153-154°C. Melting point according to Beilstein, 154-155°C.

Soil Perfusion Technique.

Lees and Quastel (64, 65, 66, 100,) evolved a soil perfusion technique for the study of nitrogen and other metabolism by whole soils. With slight modifications, this technique was adopted as the principle method of studying the breakdown kinetics of the various compounds in contact with moist soil. In this technique, a fixed volume of solution, of the compound under test, was automatically and continuously circulated through a fixed amount of normal soil. The soil solution was also aerated by the circulation. In this way the fate of the test compound was determined with an accuracy limited only by the assay methods available. Lees and Quastel showed that, in biological processes in soil, the time/progress curve was of characteristic shape. An initial lag was followed by an exponential rise; characteristics which were typical of bacterial proliferation in pure culture. Such curves obtained from perfusions may be regarded as strong evidence of similar bacterial proliferation in the soil, in response to the specific metabolite employed. Succeeding these initial stages, a fairly steady maximum decomposition rate was usually achieved, when the soil may be regarded as having been "saturated" with a stable, equilibrium population of the particular bacterial, or other micro-organismal, species responsible for the degradation of the metabolite in question. The behaviour of such a stable population of organisms, adapted to one compound, when presented with a second compound, was easily studied by draining the first solution from the perfuser and refilling

with a solution of the second compound.

(a). The Perfuser.

Audus (6,) had already modified and simplified the original Lees and Quastel (64, 65, 100,) perfuser, but further minor modifications, designed to ensure more or less trouble free running over long periods, were introduced (Diagram I).

The soil was held between loose glass-wool plugs in a glass tube A (10" x 1") and the perfusate in the 300ml. separating funnel B. In the Audus perfuser, suction was applied to the top of the soil column through a further glass-capillary resistance tube as well as to the top of the separating funnel. In the simplified form, suction was applied only to the top of the separating funnel. The flow rate was controlled by the tightness of a cotton-wool plug in the tube (D) between the suction line and the perfuser. The base of the separating funnel and the top of the soil tube were connected by an external system of glass tubes similar to the original arrangement. The uprising tube (E) was found to function most efficiently if approximately 4mm. in diameter (internal). The lower U-tube (F) was made from tubing of 6mm. bore because (a) it had greater mechanical strength, and (b) it had lower impedance to the flow of perfusate between the separating funnel and the insertion of the side-arm. During the course of a perfusion, the resistance of the soil column to liquid flow tended to increase due to (a) some loss of crumb structure in the soil

column, and (b) the accumulation of a slimy material, possibly micro-organisms or their products, especially in the glass-wool plugs. The effect of this increased resistance was to increase the difference, at pressure equilibrium, between the liquid levels in the separating funnel and in the external tube system. When the level in the external tube system, at equilibrium, with suction on, fell below that of the side-arm insertion, the perfuser ceased to function. In extreme cases the resistance of the soil column became so high that a detached liquid column could not be drawn up the uprising tube (E) and instead, air was drawn back round the U-tube from the side arm and entered at the base of the funnel. A fairly effective way to counteract the increasing resistance of the soil column was to ensure a good difference in level, at the start, between the liquid surface in the separating funnel and the point of insertion of the side arm. Sucking back was prevented by making the U-tube deeper and cutting down the air flow rates, by manipulating the cotton-wool resistance plug. From the liquid surface in the separating funnel, 18ins. to the base of the U-tube, and 8ins. to the point of insertion of the side-arm, respectively, were found to be quite adequate distances under normal conditions. Lowering the side-arm insertion to this point introduced a mechanical difficulty, for the open end of the side-arm had to be kept higher than the liquid level in the separating funnel. If not, suction failure would have resulted in loss of perfusate from it. A simple side-arm, as on the Audus perfuser, would on account of its length, have been too heavy and unsafe, especially when

filled with liquid. To overcome this difficulty the side-arm was modified as shown in the diagram.

The method of releasing suction from a perfuser, when it was desired to take a sample from it, was also modified. In the Audus perfuser suction was released by slipping off the rubber connection to the suction line. This proved an inconvenient and risky procedure with the long bank of closely set perfusers used in these experiments. To overcome the difficulty, connection to the suction line was made through a T-piece (H), the front end of which was closed by a short piece of rubber-tubing and glass-rod (G). Suction was easily released by withdrawing the glass-rod.

In order to prevent the soil and glass-wool from settling down in the tube, and blocking the exit hole, a small tripod (J), made from thin glass-rod, stood in the bottom of the soil tube.

(b). Setting up, and routine management of, a Perfuser.

Each perfuser was completely assembled from the thoroughly cleaned parts, with the small glass tripod and two glass-wool plugs in position. It was then broken down into the minimum number of sub-assemblies (usually 2 or 3) required in order to get it into an autoclave, or steamer. Often, disconnection of the U-tube was all that was required. Sterilisation was achieved by steaming for one hour. No more drastic treatment was required as sporing organisms were not thought to participate in the breakdown of the substances under test. After cooling, in the steriliser, each perfuser was reassembled as quickly as

possible to prevent contamination. 50gms. of the sieved, dried soil was then placed in the tube between the glass-wool plugs. 250mls. of the test solution was poured into the perfuser via the side-arm. It was found that sterilisation of this solution, and cooling prior to adding it to the perfuser, had no effect on the subsequent behaviour of the perfusion and adaptation phenomena. This is understandable for each solution was freshly prepared, by dilution with glass-distilled water, from a sterile stock solution and hence, unlikely to contain many viable organisms.

The rate of perfusion was governed by the fall in pressure between the open end of the side-arm and the suction line and the permitted air flow rate. With a fixed pressure drop the rate was determined for each individual perfuser by the degree of tightness of the cotton-wool plug between perfuser and suction line. By adjustment of the tap at the bottom of the separating funnel it was possible to produce a continuous stream of small water and air columns in the uprising tube rather than spasmodic long ones. The total carry over was not much affected by this adjustment, only its character. The rates of flow aimed at were approximately 1 to 2 litres of perfusate and 5 litres of air per hour. Perfusers were found to operate more reliably, over long periods, with flow rates of this order. The rates were not critical values as far as adaptation phenomena were concerned, for lag period and breakdown rate, for any given compound, seemed to be unaffected by perfusion rate. The rate of perfusion invariably decreased as the perfuser aged,

due to increasing resistance of the soil column.

Perfusers set up in this manner often ran with little trouble for 200 days and occasionally for over 300 days.

Sampling the Perfusate.

Individual perfusers were easily stopped for sampling by taking out the glass plug at the front of the T-piece. The perfusate rose in the side-arm and eventually found its own level.

The solution strength used in the perfusers depended chiefly on the nature of the substrate in question. 100 parts per million was the most commonly used concentration, though perfusions were also carried out with 2,4-D concentrations as high as 1,000ppm. and with 2,4,5-T, etc., as low as 10ppm. Frequency of sampling and size of sample, or samples, for any perfuser depended on (a) the expected rate of disappearance of the compound, (b) the concentration of the compound in the perfusate, and (c) the final concentrations required for assaying the compound.

Samples were taken with pipettes which had been freed from adhereing herbicide and adapted organisms by standing, immersed in alcohol, for a minimum period of 15hrs. The alcohol was removed by washing with tap water and rinsing with distilled water. The pipettes could not be regarded as sterile but, as the results were no different when sterile pipettes were used, sterilisation was regarded as an unnecessary precaution. There is some tendency for herbicidal substances to adhere to glass so, before the actual sample

was removed, the pipette was first equilibrated by filling several times with perfusate and allowing it to drain back into the side-arm. The samples were pipetted directly into labeled test-tubes (dry, or containing a small amount of water, depending on the final assay concentration required) and sterilised by standing in a boiling water-bath for a minimum of 30mins.

After sampling, the perfuser was restarted by reinserting the glass plug.

When it was desired to recharge the perfuser with substrate, two courses were available, (a) to add the required amount of concentrated stock solution to the perfusate via the side-arm and make up to the required volume with a little distilled water, or (b) to drain the old perfusate as far as possible and refill with fresh perfusate of the required strength. This second method was sometimes varied by washing out the whole perfuser with one or two changes of distilled water before refilling. The non-drainage method could have led to the accumulation of toxic degradation or waste products. Toxic effects of this kind were never observed and the only real drawback of the method was the relatively long time needed for the perfusate to reach a uniform concentration. The drainage method no doubt entailed the loss of water-soluble substances, and adapted micro-organisms, from the soil but did not result in loss of activity. There was no detectable difference in the course of the perfusion whichever method was used. The drainage method was most commonly employed.

In order to drain the perfuser, the separating funnel tap was

closed and as much liquid as possible allowed to drain from the soil with suction still on. Suction was then released by removing the glass plug, the U-tube disconnected and emptied and the separating funnel drained. The U-tube was then reconnected. 250ml. of fresh solution was poured into the perfuser, through the side arm, with suction on once again. About 1hrs. perfusion was allowed, before sampling, in order that the fresh perfusate might become thoroughly mixed with the liquid retained by the soil. It was found that 50gms. of the soil used retained almost exactly 50mls. of liquid, against normal drainage. Thus, the total volume of perfusate after normal draining and refilling was very nearly 300mls. When only qualitative results were required, this factor did not matter, but for determining the breakdown rates quantitatively, allowance would have to be made for the dilution factor. Attempts were made to speed up the equilibration of the fresh solution with the soil retained liquid by adding the fresh solution directly to the top of the soil column rather than through the side-arm. The effect of this sudden waterlogging of the soil was to cause a more rapid deterioration of the soil crumb structure and a consequent decrease in the useful working life of the perfuser. The method was abandoned in favour of the side-arm refill and one hours equilibration. Some assay results, and more particularly phenol determinations suggested that equilibrium was not always achieved in one hour One hour was chosen as a compromise, for any advantage of delaying the sampling might have been offset by a measureable change in substrate concentration, especially with a very

active perfuser.

After the initial lag phase, followed by the first disappearance of the substrate, the perfuser was refilled on several successive occasions, with the same substrate, till the maximum breakdown rate was achieved. The soil column was then regarded as "saturated" with the micro-organisms responsible for the breakdown. In this state its behaviour resembled that of a pure culture or even that of a single metabolic unit. Its activity towards compounds related to the initial substrate was then determined, by draining and refilling the perfuser with a solution of a second substrate.

Many successive perfusions, of two or more substrates, were studied in this way, as well as perfusions of single substrates, mixtures of two or more substrates, etc.

Accelerated enrichment of fresh soil by perfusate from an enriched perfuser.

This technique proved very valuable for producing enriched perfusers without having to endure the lag phase which in some cases would have been very long and time wasting. When the substrate had disappeared from an active perfuser, the perfusate was drained out into a sterile flask and made up to 250ml. of the required concentration by adding distilled water and the correct amount of concentrated stock substrate solution. This "active" solution was then poured into a new perfuser in the usual way. It was found that there was still a slight lag before breakdown commenced but that this was much shorter than the primary lag of a new non-activated

perfuser. The lag phase of 2,4-D was reduced from about 20 days to 5 to 10 days, while that of MCPA was reduced from 70+ days to approximately 30 days. In these cases the saving in tim time was of great value but in the case of 4-CPA which had a normal, short lag phase of about 10 days, direct enrichment was the rule. As well as the saving in time, the accelerated enrichment method allowed the production of two, or more, active perfusers which were more alike than could have been obtained by adapting each one separately. The active perfusate was simply made up to some multiple of 250ml. and divided between the required number of perfusers.

An interesting discovery was that although enrichment of the soil could be transferred in the above manner, for those phenoxyacetic acids for which it was tried, several attempts to transfer 2,4-dichlorphenol (2,4-DCP) enrichment,in the same way,all resulted in failure.

Assay Technique.

(a) Introduction.

Known methods for the assay of selective herbicides of the 2,4-D type, and related compounds, may be divided into two groups, (a) the physico-chemical and (b) the biological.

Methods have been elaborated for the qualitative and quantitative determination of 2,4-D by ultra-violet or visible range absorbtion spectrometry (12, 67,) and of MCPA by infra-red absorbtion spectrometry (39, 106,). Though these methods can be made quite specific, expensive apparatus is

required and, in the case of soil perfusates, extraction and purification of the herbicide would be required if reasonably accurate determinations were to be made.

Letourneau and Krog (67,) devised a quantitative spectrometric method for the determination of 2,4-D based on the observation by Freed (35,) that, in the presence of hot concentrated sulphuric acid, 2,4-D reacts with chromotropic acid to give a purple colouration. Many other substances, both related and unrelated, give the same colour reaction. Formaldehyde, or other aldehyde, is believed to be responsible for the colour reaction and to be produced during the heating of each positive substance with concentrated acid. Apart from a wide variety of organic compounds, soil perfusate is likely to contain nitrite and nitrate ions, two of the inorganic ions which also give a colour reaction with chromotropic acid. Though more specific, the physico-chemical methods are probably no more accurate than bio-assays and the tedious separations and purifications required, before reasonable results can be expected, preclude their use for routine determinations where large numbers of samples are involved.

The Avena curvature, Avena cylinder and Went's split pea stem tests have long been used for the assay of auxin, but it was some time before similar methods were applied to the assay of selective herbicides.

Earlier workers (2, 16, 27, 28, 42, 48, 49, 59, 62, 72, 81, 86, 89, 92, 107, 126, 131,) studying the disappearance of 2,4-D from soil used the "all or none" method of seedling response and emergence. Seeds of susceptible species such as

kidney beans, African marigolds or mustard were sown at [illegible] intervals in the soil and the emergent seedlings (if any) were examined for hormone induced malformations. In this way an approximate estimate of persistence was obtained. Fairly large amounts of soil were required and the results were qualitative rather than quantitative as it was difficult to correlate herbicide concentration in soil with effects on seedlings, especially at low concentrations. Some improvement of this technique was made by Hernandez and Warren (47, 48, 49,) who weighed seedlings (cabbage) grown for 18days, in the soil, under standard conditions.

In order to improve the reliability of bio-assays, it was realised that the herbicide would have to be extracted from the soil and assayed under more controlled conditions for it has been shown that the toxicity of a given herbicide concentration can vary in different soils (11, 92,). Ready and Grant (102,) introduced a method for extracting the herbicide from soil and determination of its concentration by its effect on root elongation in Cucurbita seedlings. Swanson (119,) described a similar method in which selected seedlings of hybrid field corn (Zea) provided the roots. Both methods have been used by workers in America, Swanson's method being one of three used by Thomson et al (125,) for the evaluation of approximately 1,100 organic compounds as growth regulators. They also used the kidney bean single drop tests (oily or aqueous solutions) in which single drops of the test solutions were applied to growing points of young kidney bean plants and the effects

evaluated. Moewus (82, 83,) described a method for which an accuracy of ± 5% was claimed. In it, selected cress seedlings (Lepidium sativum) were grown under standard conditions, on filter paper, in Petri dishes containing a standard amount of the test solution. The mean increase in root length was compared with that produced by known amounts of hormone under the same conditions. Though originally developed for the determination of hetero-auxin, the method has been applied successfully to synthetic growth regulators. A similar, but simpler, method is that described by Audus (9,) and based on an earlier method by Audus and Quastel (10a,). Cress seedlings again form the test material. Though an accuracy of approximately ±10% is all that has been claimed for the method (cf. Moewus claim of ±5%), its slightly higher sensitivity and greater simplicity made it the obvious choice for the present research. It would have been impossible to carry out the large number of assays involved if a more accurate and refined, but more laborious, bio-assay method had been used. This would have been especially true if the method required pre-growth and selection of seedlings (eg. Moewus and Swanson methods). Some detail modifications to the Audus test were made, but the basic principles remained unaltered.

(b). Cleaning pipettes.

Immediately after use, all pipettes were thoroughly washed in boiling water then stored, submerged in alcohol, for a minimum period of 15hrs. before

further use. When required, they were removed from the alcohol and washed by running tap water through each one for about 15secs. After further washing with glass-distilled water they were dried on the outside with a clean cloth and allowed to drain dry before use.

(c). Cleaning assay tubes.

It was found that traces of adsorbed hormone, in assay tubes, could cause very erratic results especially if tubes used once at higher concentrations were subsequently used for lower ones. Ordinary soap and hot-water washing was ineffective in removing adsorbed material and it was thought that adsorbed synthetic detergents might also produce spurious results. An elaborate technique was evolved which could be carried out fairly easily and gave good results providing the alcohol was changed frequently.
Tubes used for the assay were rimless Pyrex test-tubes (19 x 150mm.). After use they were scrubbed out individually and rinsed in hot water. They were then packed into wire baskets and the baskets completely immersed in alcohol in a covered glass tank. Soaking was continued for at least 15hrs. to ensure as complete a removal of herbicide as practicable. After this, the baskets of tubes were removed, as units, and the contained alcohol returned to the tank. Each unit was then washed thoroughly in running tap -water till all traces of alcohol were removed. After draining off the tap-water, 5 to 10mls. of glass-distilled water was added to each tube, the tube shaken vigorously to rinse it, then stood upside down in

another wire basket, to drain. The baskets of clean tubes were then maintained at 150°C for 1 hr. in a hot-air oven. In this way the tubes were dried and, for the purpose of the assay, could be considered sterile. Only certain sporing organisms might withstand this treatment and as they were unlikely to be present and are not thought capable of attacking the herbicide molecule, this eventuality was disregarded.

For a time, tubes were soaked in alcoholic soda, in place of alcohol, rinsed in water then in dilute hydrochloric acid before proceeding to the final rinsing stages. The tubes did not appear to be cleaner, nor the results better, for the extra effort and this procedure was abandoned in favour of the simpler one.

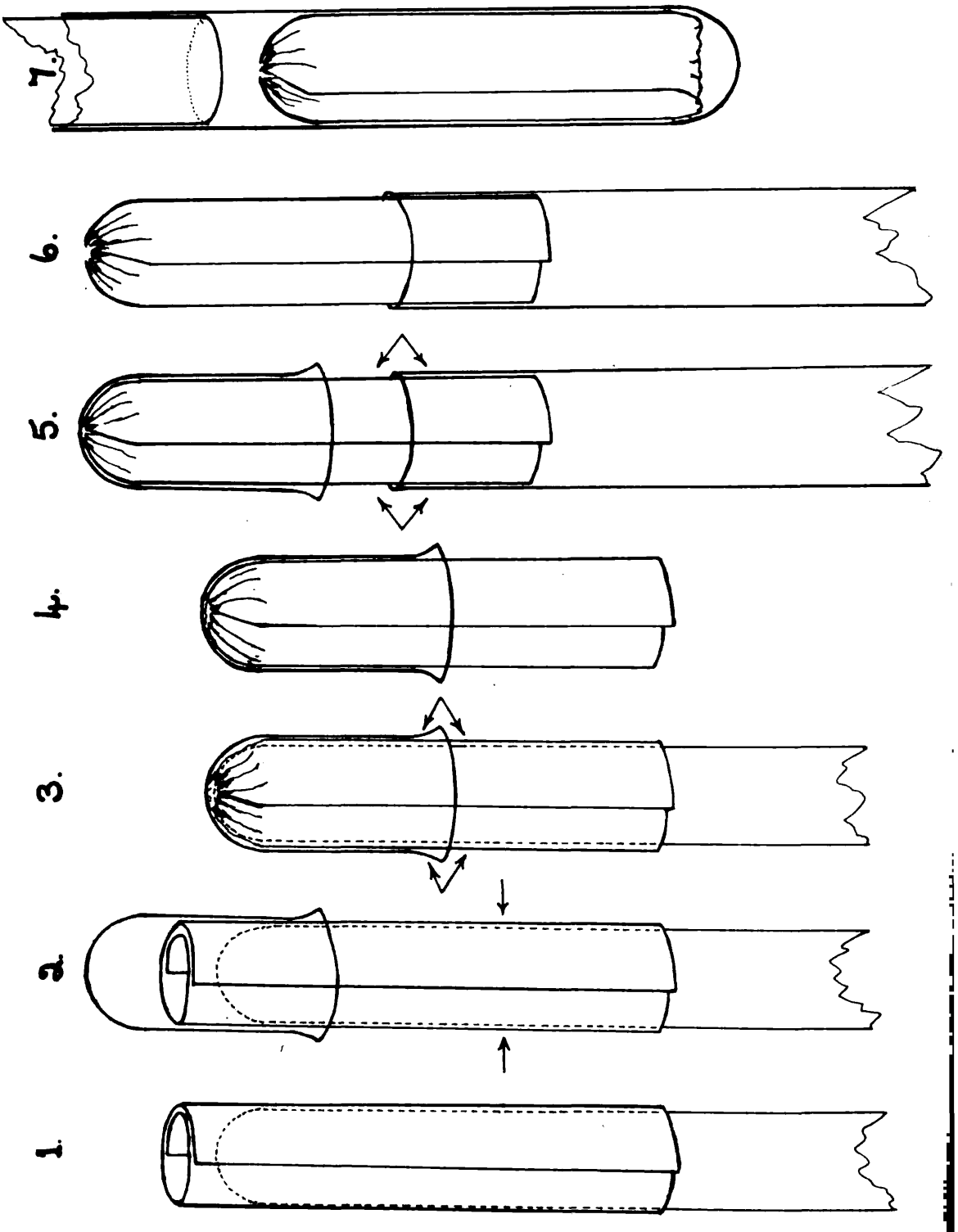
(d). Paper cylinder supports for the seeds.

In order to give the cress roots good aeration and yet surround them with the test solution, Audus (9,) supported the seeds on a 8cm. high filter paper cylinder, fitting closely inside a test-tube and topped by a separate filter-paper cone of the same basal diameter. The seeds were supported in the annular groove between cone and test-tube. They readily absorbed water when the paper was moistened. The emergent radicles grew down vertically between the paper cylinder and the glass, receiving an adequate supply of water and showing no signs of oxygen deficiency.

The cutting, folding and fitting of the paper cones proved to

be too laborious and time consuming when large numbers of assay tubes were required. A faster, simpler and more effective method was therefore devised. A cylinder with a domed top was pressed in one piece from a filter-paper square (8cm.). For the purpose of the modified method the assay tubes were carefully selected so that their diameters all fell within the range D $\pm$ 0.5mms. D was about 19mm. but its actual value was unimportant so long as the narrow diameter range was maintained. One of the tubes from the lower limit of the range was taken and its closed end made as near hemi-spherical as possible by softening in a blow-torch flame and carefully pressing out irregularities, from the inside, with a waxed tube of smaller diameter. The end was then carefully annealed and cooled. This tube was cut to a length of about 4cm., the edges rounded off in a flame and the mouth reamed out slightly. It will be refered to as"outer tube A".
An"inner tube B" was also selected, of thick glass and with a hemispherical end. The diameter of this tube was such, that when an 8cm. filter-paper square was wrapped round it, it was an easy sliding fit into "outer tube A". The relationship between the external diameter of "inner tube B" and the internal diameter of "outer tube A" proved to be an important factor. If the fit was too tight, there was a danger of bursting "tube A" during the "paper making" process. At best it proved difficult to get"tube B" and the paper cylinder out again. If "tube B" was too narrow, badly formed, wrinkled cylinders were produced with poor domed tops. These were difficult to fit into the assay tubes proper. The actual

DIAGRAM. II.



process for making the domed-topped paper cylinders was as follows.

An 8cm. filter-paper square (Whatman No.2.) was wrapped tightly round the "inner tube B" with about 0.75cm. projecting past the tip (Fig.1.). The paper was gripped tightly to the tube at the points indicated by the arrows (Fig. 2.) and the "outer tube A" placed in position as shown. The tip of "tube A" was then placed against a padded rigid surface and,while still gripping the paper to it, "tube B" was forced into "tube A". This caused the free end of the paper cylinder to be compressed into a domed shape between the two hemispherical glass surfaces (Fig.3.). The "outer tube A" and the paper cylinder were held at the points indicated by arrows in Fig.3. and the "inner tube B" removed (Fig.4.). The open end of the paper cylinder was then placed in the mouth of an assay tube (Fig.5.). Assay tube and paper cylinder were gripped at the indicated points and the"outer tube A" removed (Fig.6.). The domed paper cylinder was then pushed down to the bottom of the assay tube (Fig.7.) using the open end of another test-tube of diameter small enough to allow it to just slide easily into the smallest assay tube of the range. The quality of the domed tops produced by this method depended,to some extent on atmospheric conditions, particularly on relative humidity. The paper squares were most sensitive for, when too dry, the domed tops tended to open out and, when too damp, it was often impossible to get a completely closed top and difficult to get "tube B" out of, or "tube A" off, the paper cylinder.

The state of the inner surface of "outer tube A" also had a marked effect. After several dozen paper cylinders had been made, the quality usually deteriorated but could be restored by gently heating "tube A" or, better, by polishing the inner surface with a clean cloth. Occasional polishing of "tube B" made it easier to remove at the stage Fig.3 to Fig.4.

(e). The cress seeds (Lepidium sativum).

The best variety for the assay was found to be "Carter's Plain" (Messrs. Carter's Tested Seeds Ltd., Raynes Park, Wimbledon, London.). This variety had a consistently high germination rate under the assay conditions and a high percentage of long straight roots. It would appear that cress seeds need to undergo some maturation process for they behaved much more consistently when at least one year old (from harvesting time). Such seeds produced roots of 80 to 100mm. long, or more, under the conditions of the test.

As in the Audus method (9,) , 20 seeds were used per tube. To eliminate tedious hand counting, a scoop was made by sealing a short length of glass tube (approximately 5mm. long by 4mm. internal diameter.) onto the end of a glass rod. This scoop held 20 $\pm$ 2 seeds and proved very satisfactory for 20 seeds was not a critical value. Apart from its quickness, use of the scoop eliminated any tendency to unconscious selection of the seeds. The seeds were kept in a loosely covered box with free access to the air and were scooped as required into

the assay tubes. The 20 seeds were distributed fairly uniformly round the circumference of the paper dome by gently tapping the tube.

(f). The assay procedure.

From the sterilised sample, or samples (taken as described on p.26), a series of three dilutions was prepared for each assay. The two higher dilutions were 1/10 and 1/100 of the highest concentration to be used. These three concentrations were nominal fractions of the initial herbicide concentration placed in the perfuser and the chosen values depended upon the herbicide in question. The nominal concentration values were chosen after consideration of the standard concentration/growth curve for the herbicide and were such that the stronger of the three dilutions (assuming no breakdown to have occured) would cause almost complete inhibition of the cress roots, while the weakest (1/100th. as strong) would permit control, or near control, growth. The intermediate concentration allowed growth in the region of 50% of the control level.
2,4-D and 2-CPA may be taken as examples to illustrate the principles of the dilution technique.
(i). 2,4-D causes almost complete inhibition at 1ppm. and permits almost control growth at 0.01ppm. Nominal concentrations of 1, 0.1, and 0.01ppm. were therefore chosen for the assay. When the initial concentration added to the perfuser was 100ppm., it necessitated dilution of the initial 1 ml. sample by 100, 1,000 and 10,000 times to produce the

required assay concentrations.

(ii). 2-CPA allows almost control growth at 0.1 ppm. though only 10 to 30% at 10 ppm. Nominal assay concentrations were therefore set at 0.1, 1.0 and 10 ppm. With an initial perfusate concentration of 10 ppm., the assay concentrations were obtained from three separate samples. 10 ppm. by a direct 10 ml. sample, 1.0 ppm. by a 2 ml. sample diluted to 20 ml. and 0.1 ppm. by a 1 ml. sample diluted to 100 ml.

For preliminary experiments the dilutions were prepared, as in the Audus method, by dilution in the culture medium of Nutman et al (92,). This medium had been modified by the addition of a full range of accessory elements (Hoagland and Snyder's A to Z solution, 52,) and adjustment to pH 6. It was found that an all-round improvement in growth was obtainable if glass-distilled water was used, in place of the culture medium, when preparing the dilutions. Growth in length in all the herbicide concentrations was improved. Growth of the controls, in glass-distilled water only, was increased by 50 to 100% or more.

5 mls. of herbicide dilution was pipetted into each assay tube. As each paper cylinder was wetted, its domed top tended to straighten out. The seeds were thus gently pressed between the tube wall and the wet filter paper, providing good conditions for germination. Each assay normally consisted of 12 tubes, 2 controls containing distilled water only, 2 tubes at the highest concentration and 4 each at the intermediate and weakest concentration.

In the Audus method the tubes were loosely plugged with cotton

wool plugged to minimise evaporation. It was felt that plugging was more likely to interfere with O_2/CO_2 exchange than with evaporation and so was omitted without producing ill effects. The tubes were incubated for 4 days at 25°C in a near-saturated atmosphere in complete darkness (Audus had used a constant light intensity of about 4 foot-candles). At the end of the incubation period the tubes were removed and the longest roots measured in the control tubes and at each nominal concentration. Audus measured only the longest root in each tube of a given concentration but it was found that more consistant results were obtainable if all the long roots, at any one concentration, were measured and the top 2 or 4 (depending on the number of tubes at that concentration) taken as representative of the maximum growth attainable at that concentration. In some cases all the longer roots came from one tube but usually they were well distributed. It is considered that this variation in the method is permissable because,

(i). the controls were treated in exactly the same way.

(ii). the standard curves were constructed using the same technique.

(iii). it was easy for the roots to exhibit less than optimum growth at any concentration owing to such factors as delayed germination, slightly contaminated tubes, etc. Supra-optimal growth was most unlikely.

(iv). even if all the representative roots came from one tube it is most unlikely that they were abnormally long.

(v). the method gave fairly consistent and reliable results.

The most convenient way to measure the root length was to lay it along a glass covered millimetre scale. The limitations of the assay method did not warrant measurement to an accuracy of more than the nearest millimetre.

The longest control roots were measured in the same way. In all, twice as many representative control roots were taken as there were assays in any batch. The mean value of these control root lengths was determined.

The method of obtaining the herbicide concentration, in the sample, from the assay results was the same as that described by Audus. The lengths of the representative roots, for each concentration, were expressed as a percentage of the mean control root length. These figures were Root Growth Indices for the concentration concerned.

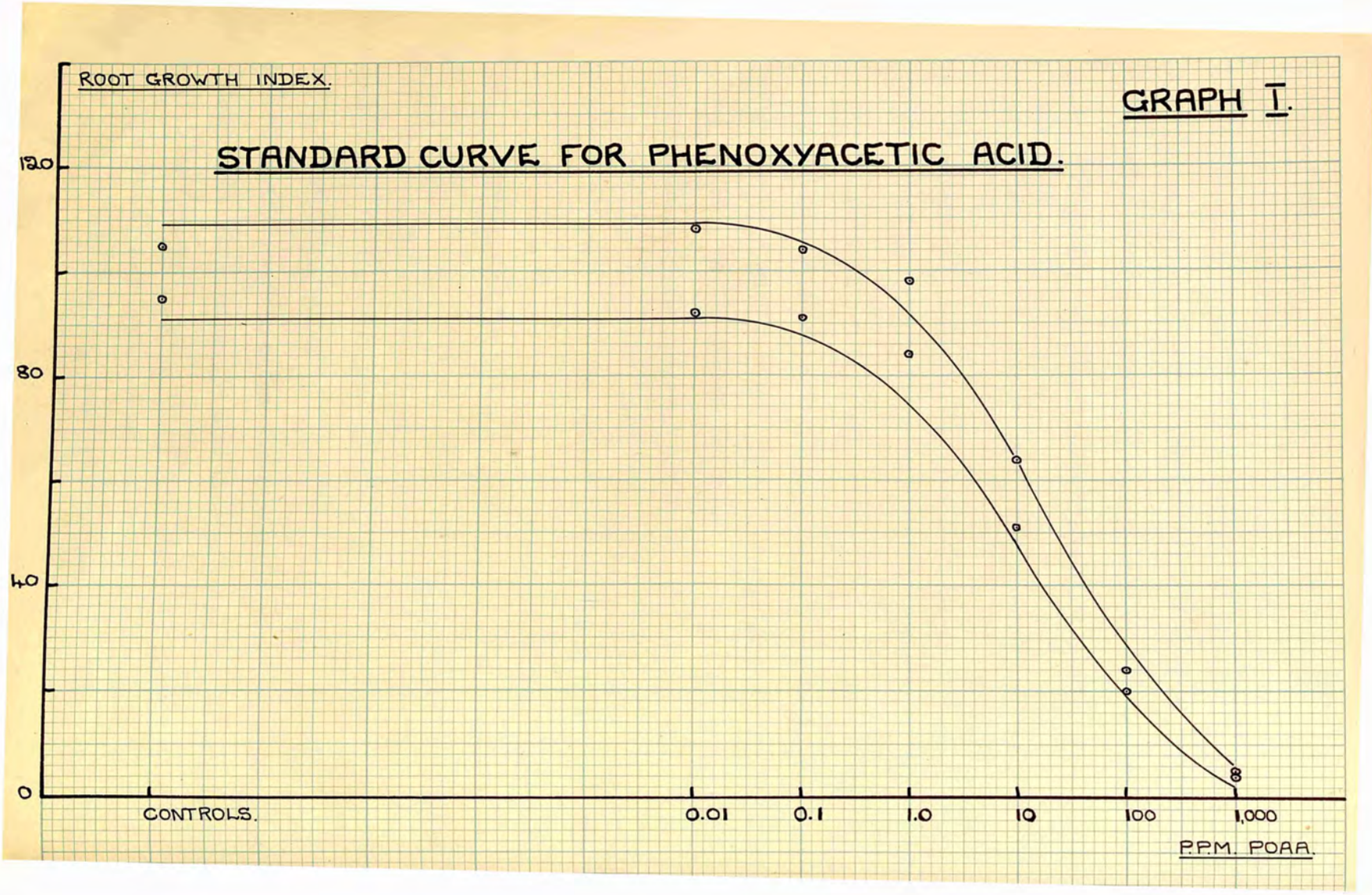
$$\text{Root Growth Index} = \frac{\text{Root length in herbicide solution}}{\text{Mean control root length.}} \times 100.$$

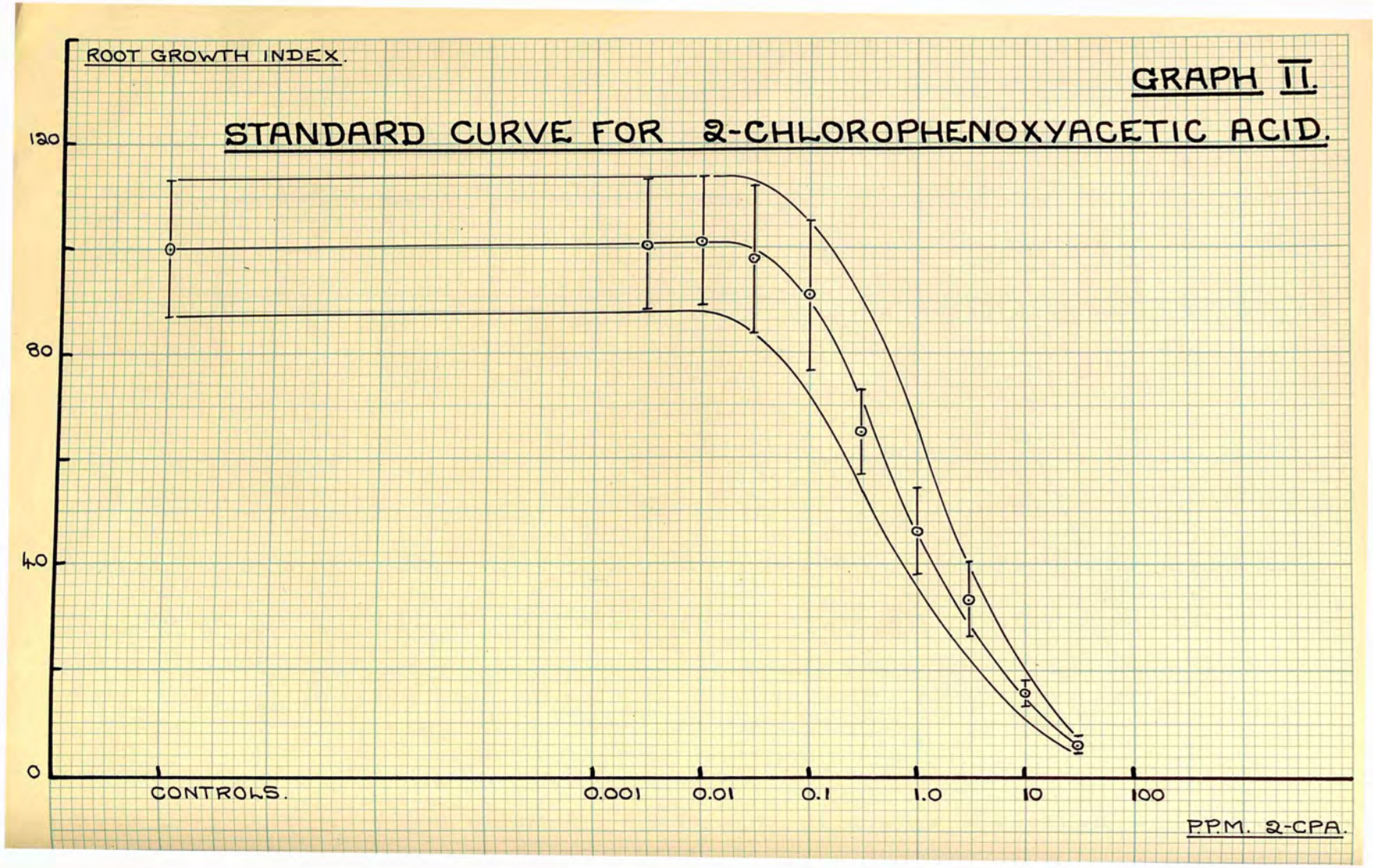
The Root Growth Indices were then plotted on a sheet of thin "Perspex" using the same co-ordinates as for the Standard Curve for the herbicide (vide infra). The "Perspex" was then placed over the Standard Curve and, by lateral adjustment along the concentration axis, the closest fit of the assay points on the Standard Curve was found. The actual herbicide concentration in the sample was then read off directly from the Standard Curve concentration axis. Position of closest fit of assay points on the curve was largely a matter of personal judgement and this constituted the weakest point of the technique. Often the assay points fitted equally well over a range of concentrations rather than at one specific concentration. In these cases the probable position of best

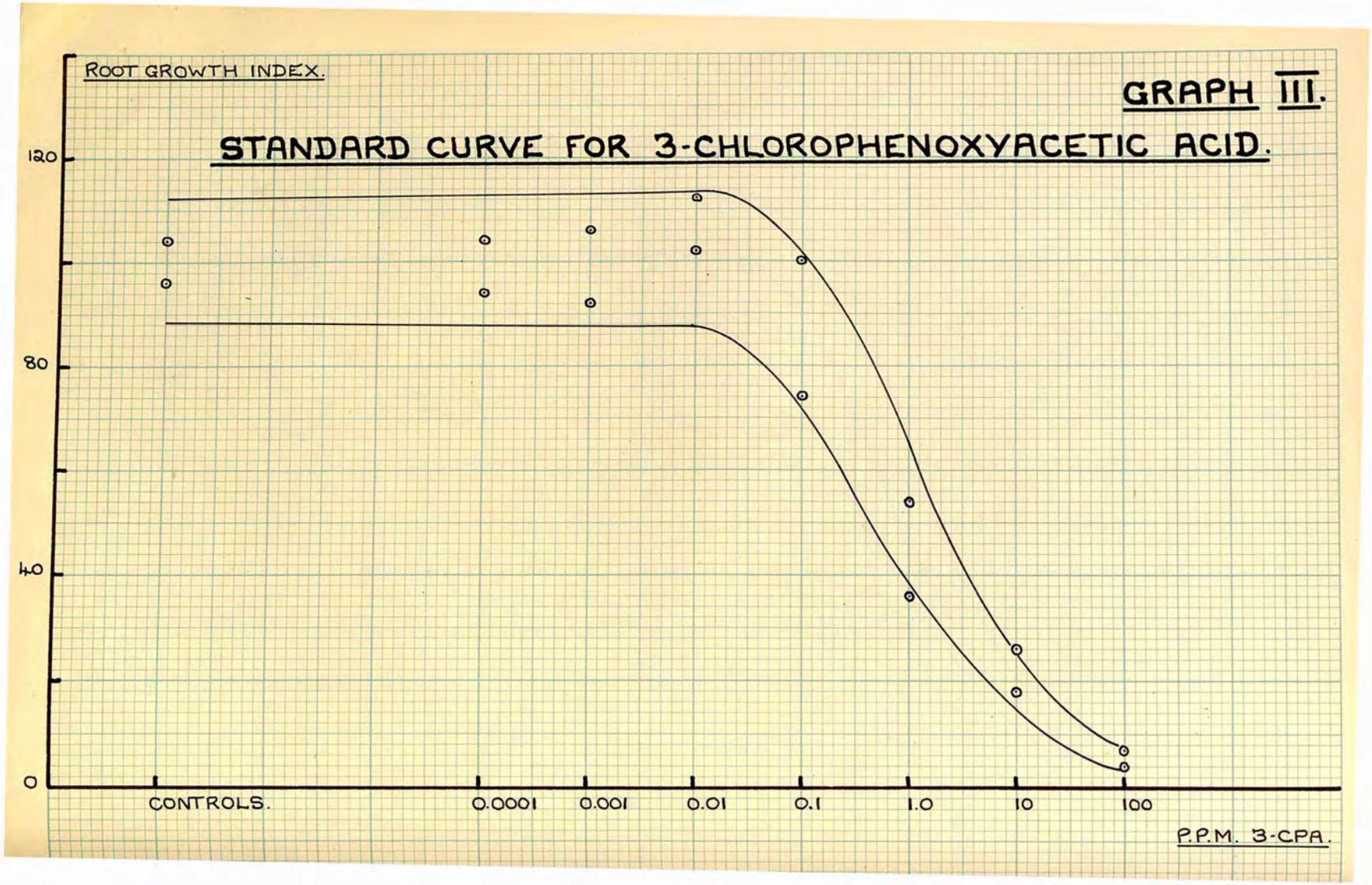
fit (and hence of herbicide concentration) was best determined after consultation of the preceeding and succeeding samples from the same perfuser. A common source of trouble was apparently stimulated growth in the highest dilution used, ie. growth greater than could be expected from pure herbicide at a concentration indicated by the assay results with the other two, higher, concentrations. The stimulation was probably due to herbicidal breakdown products, or substances naturally produced in the soil, and were only capable of producing effects when the herbicide concentration had been diluted to a point of low, or no, toxicity. These substances would not be in the pure solutions of herbicide used for standardisation where, consequently, relatively less growth resulted. Newman et al (90,) and Audus (7,) also reported the possibility of a stimulatory breakdown product of herbicides by bacterial cultures.

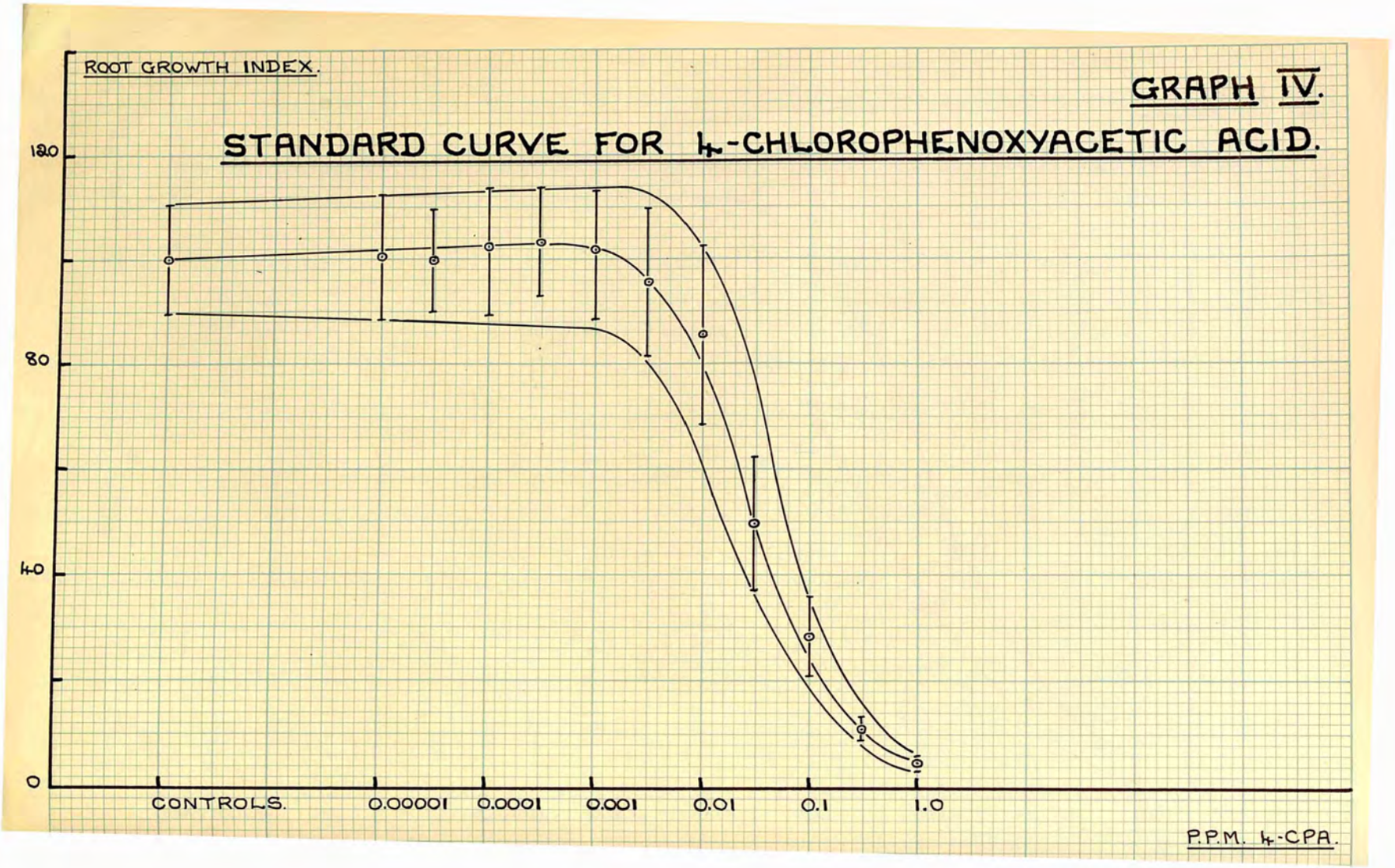
(g). Standard Curves.

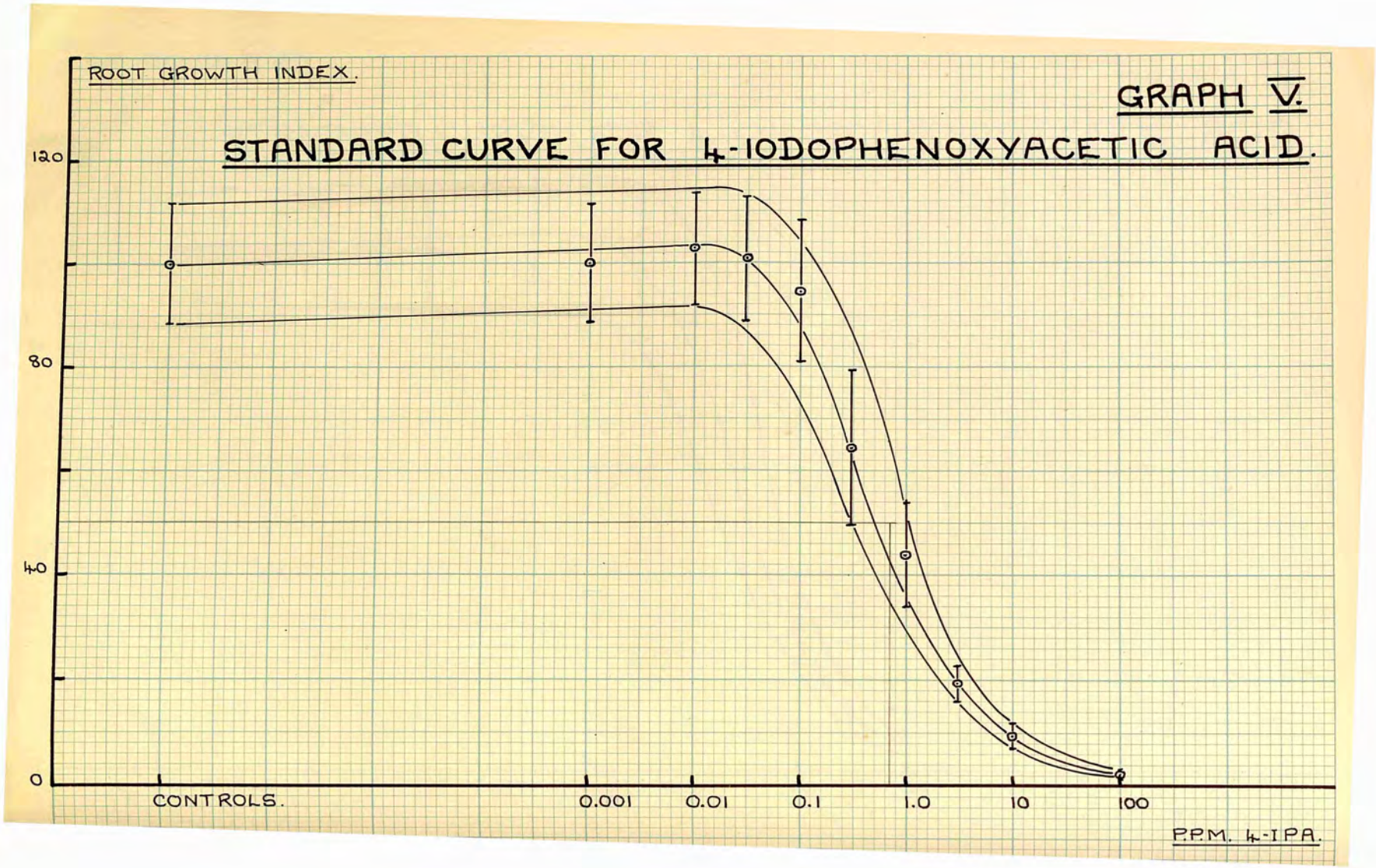
A Standard Curve of Root Growth Index against herbicide concentration was prepared for most of the herbicidal compounds tested. Concentrations of the herbicide covering the range from complete inhibition to control growth were used in each case. The various concentrations were produced by serial dilution from a stock solution (normally 1%) using distilled water as the diluent. Great care was taken to ensure thorough mixing at each stage and no "carry over" from high to much lower concentrations. Stock herbicide solutions were prepared by weighing the required amount of

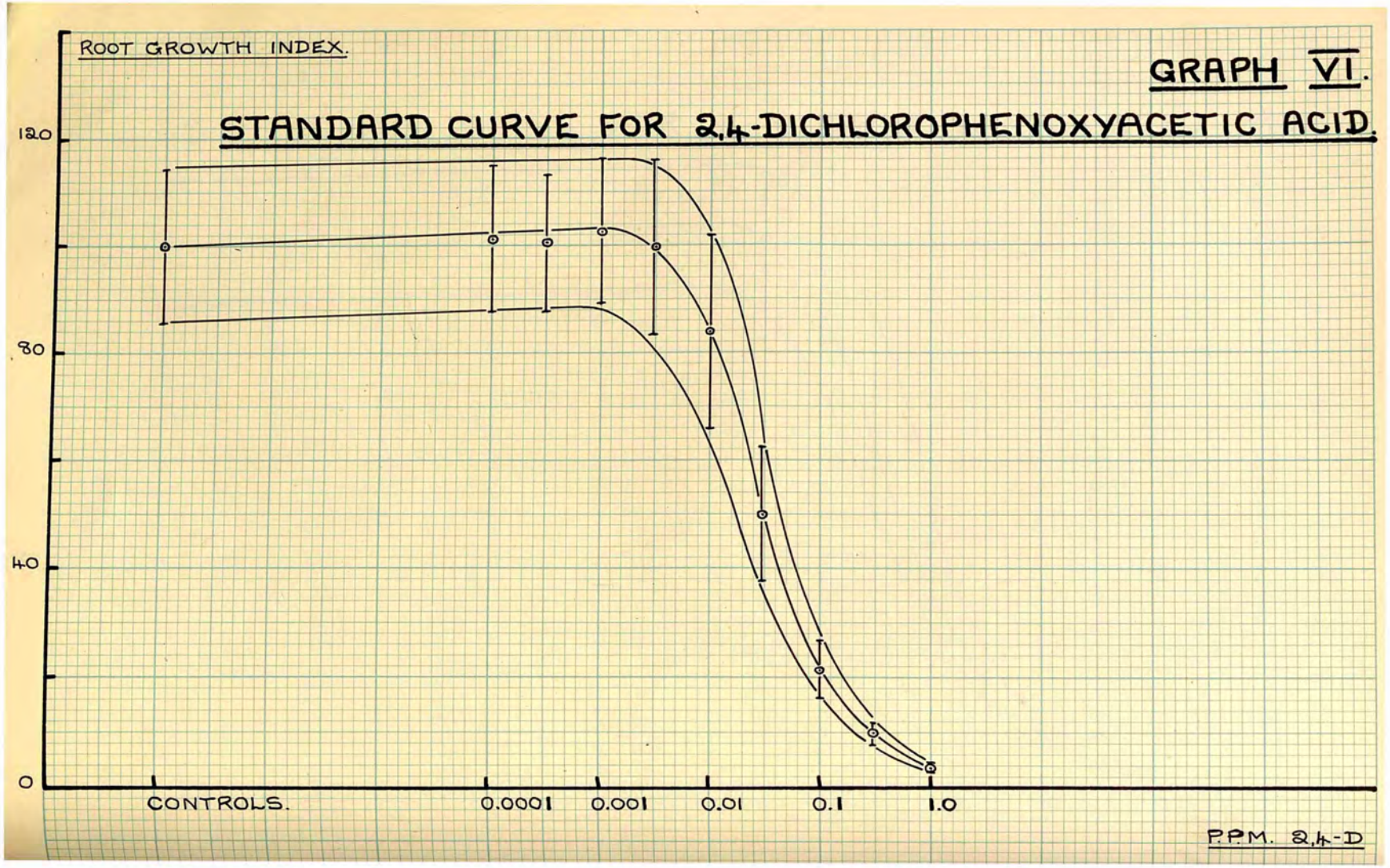

ROOT GROWTH INDEX.
GRAPH I.
STANDARD CURVE FOR PHENOXYACETIC ACID.
120
80
40
0
CONTROLS.
0.01
0.1
1.0
10
100
1,000
P.P.M. POAA.

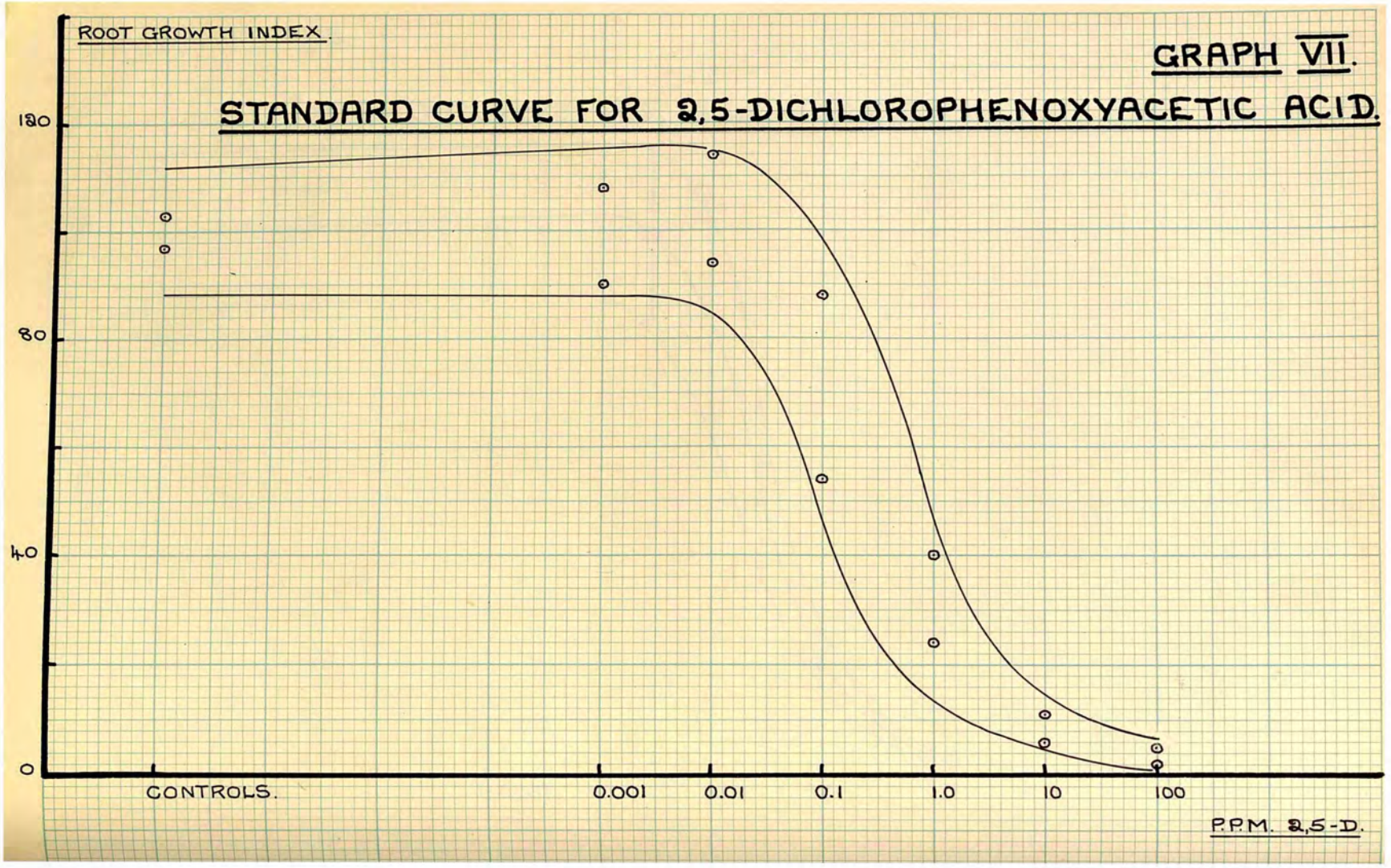

ROOT GROWTH INDEX.
GRAPH II.
STANDARD CURVE FOR 2-CHLOROPHENOXYACETIC ACID.
120
80
40
0
CONTROLS.
0.001
0.01
0.1
1.0
10
100
P.P.M. 2-CPA.

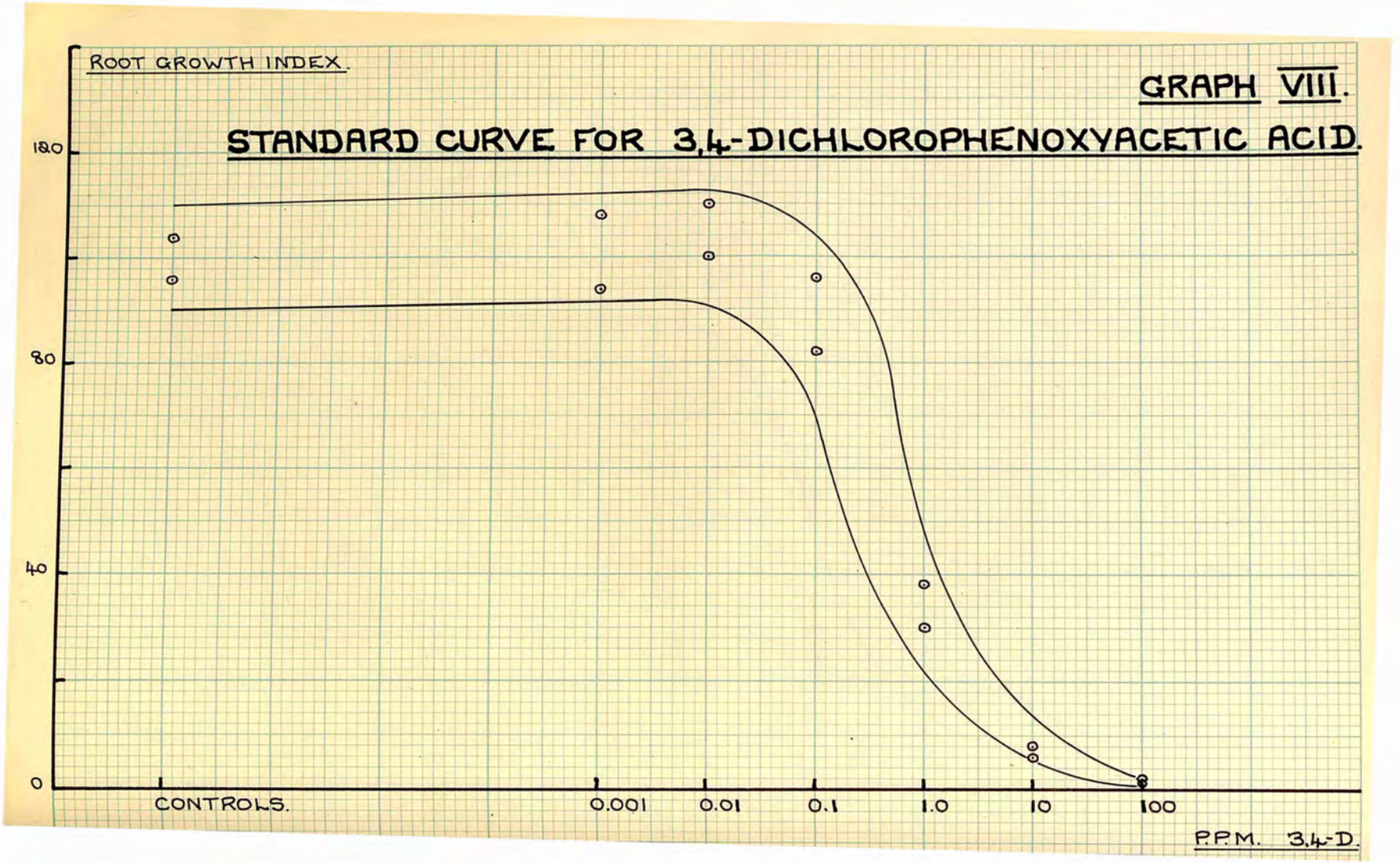

ROOT GROWTH INDEX.
GRAPH III.
STANDARD CURVE FOR 3-CHLOROPHENOXYACETIC ACID.
120
80
40
0
CONTROLS.
0.0001
0.001
0.01
0.1
1.0
10
100
P.P.M. 3-CPA.

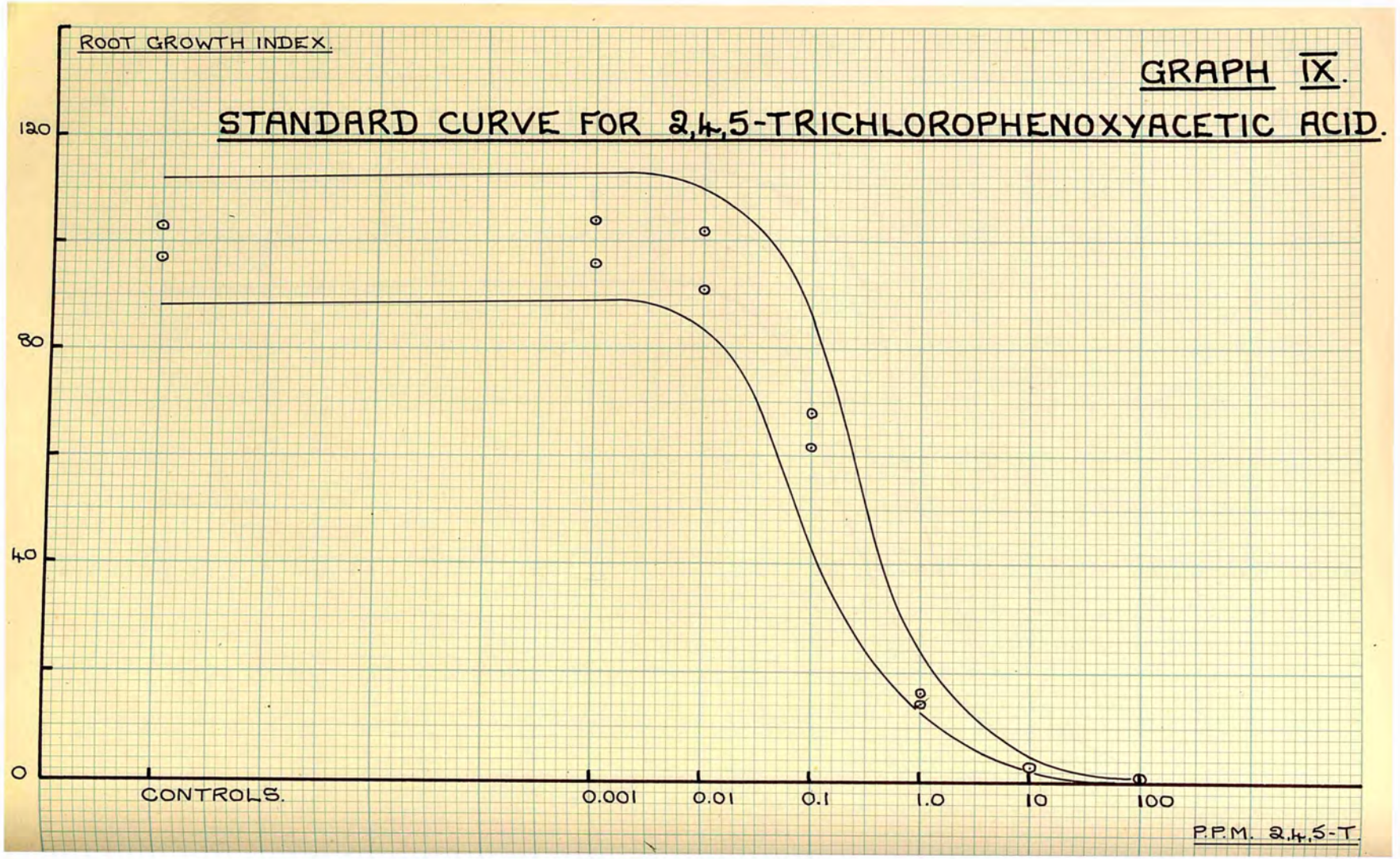

ROOT GROWTH INDEX.
GRAPH IV.
STANDARD CURVE FOR 4-CHLOROPHENOXYACETIC ACID.
120
80
40
0
CONTROLS.
0.00001
0.0001
0.001
0.01
0.1
1.0
P.P.M. 4-CPA.

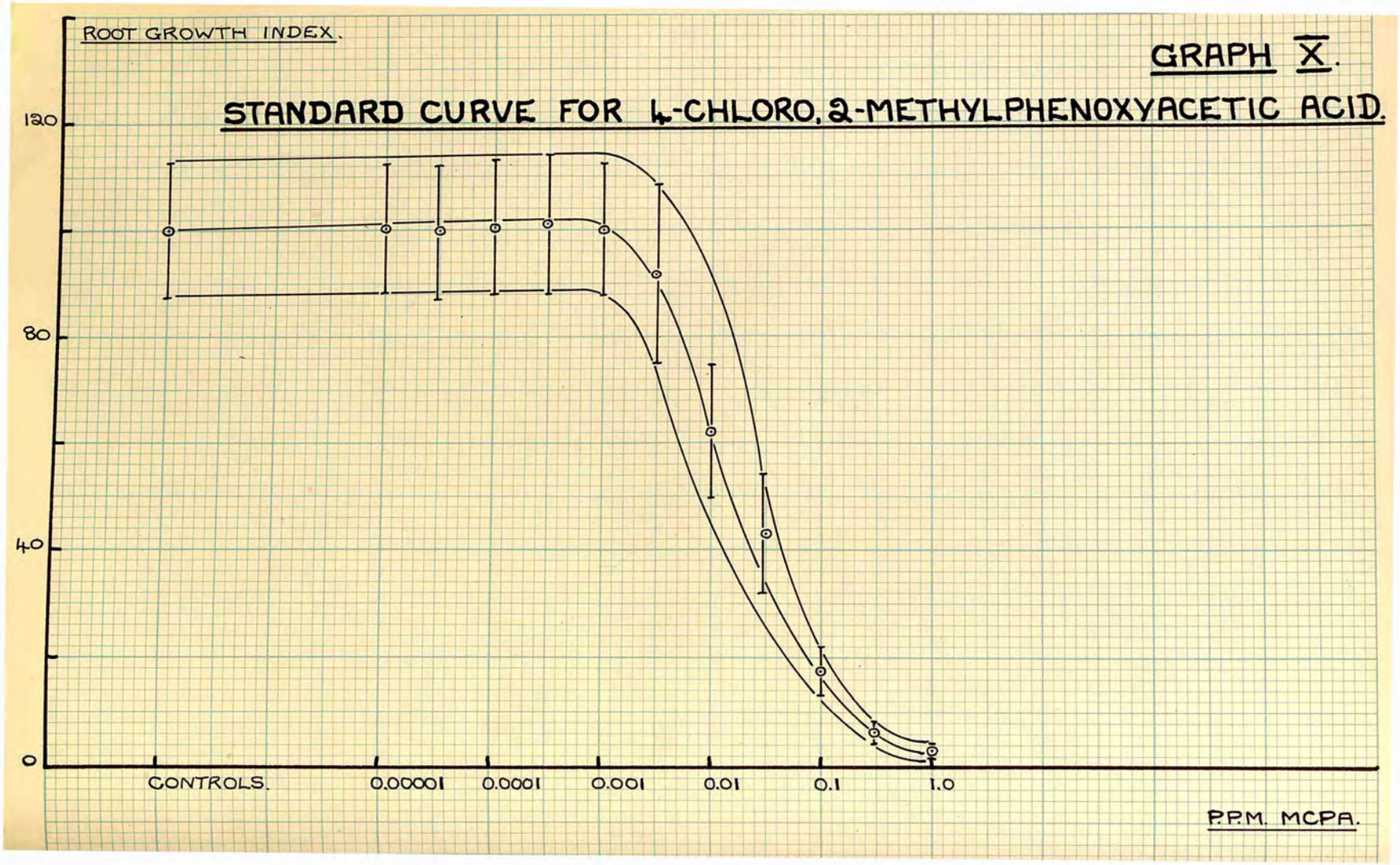

ROOT GROWTH INDEX.
GRAPH V.
STANDARD CURVE FOR 4-IODOPHENOXYACETIC ACID.
120
80
40
0
CONTROLS.
0.001
0.01
0.1
1.0
10
100
P.P.M. 4-IPA.


ROOT GROWTH INDEX.
GRAPH VI.
STANDARD CURVE FOR 2,4-DICHLOROPHENOXYACETIC ACID.
120
80
40
0
CONTROLS.
0.0001
0.001
0.01
0.1
1.0
P.P.M. 2,4-D


ROOT GROWTH INDEX.
GRAPH VII.
STANDARD CURVE FOR 2,5-DICHLOROPHENOXYACETIC ACID.
180
80
40
0
CONTROLS.
0.001
0.01
0.1
1.0
10
100
P.P.M. 2,5-D.

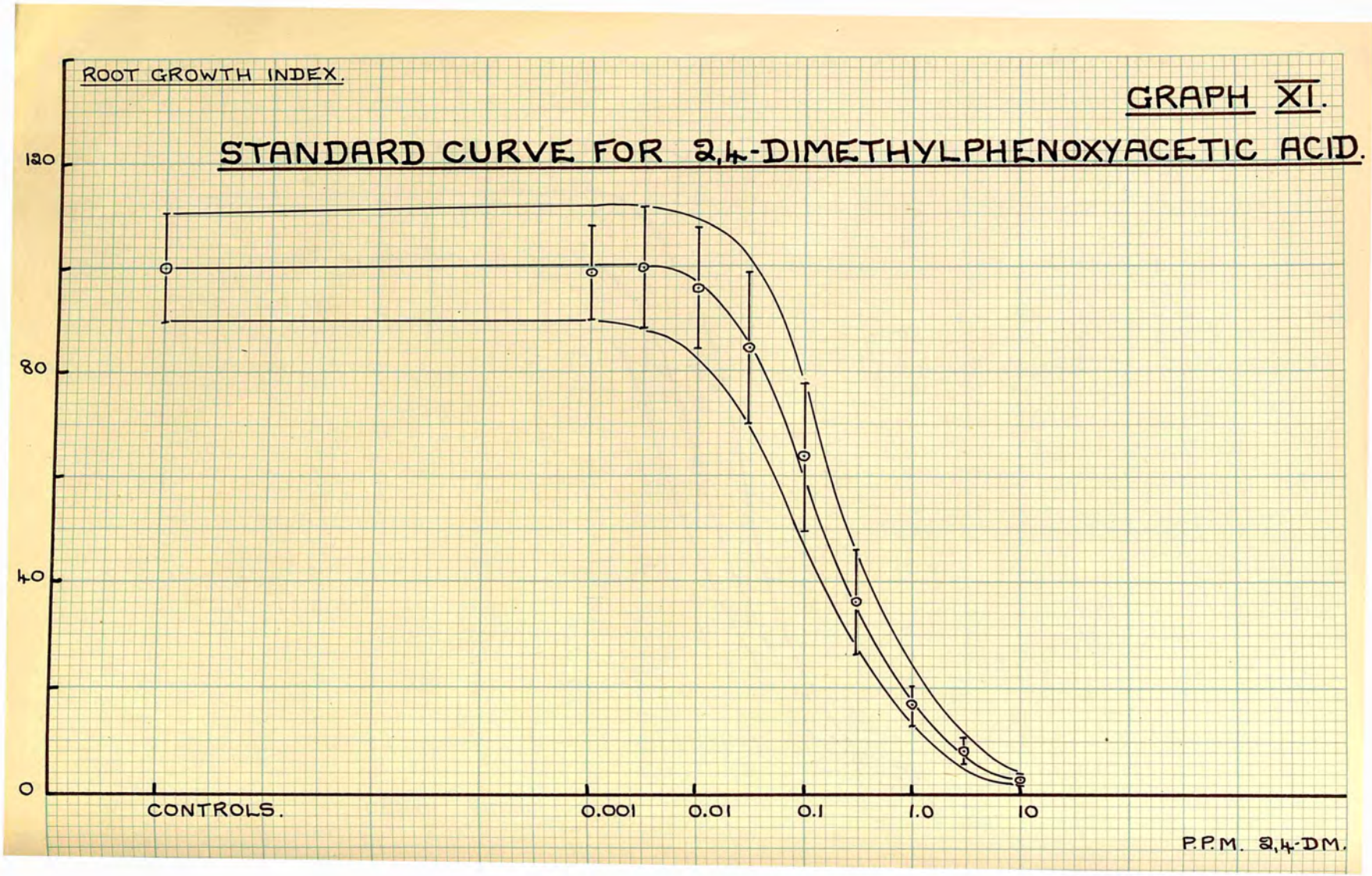

ROOT GROWTH INDEX.
GRAPH VIII.
STANDARD CURVE FOR 3,4-DICHLOROPHENOXYACETIC ACID.
120
80
40
0
CONTROLS.
0.001
0.01
0.1
1.0
10
100
P.P.M. 3.4-D.


ROOT GROWTH INDEX.
GRAPH IX.
STANDARD CURVE FOR 2,4,5-TRICHLOROPHENOXYACETIC ACID.
120
80
40
0
CONTROLS.
0.001
0.01
0.1
1.0
10
100
P.P.M. 2,4,5-T.


ROOT GROWTH INDEX.
GRAPH X.
STANDARD CURVE FOR 4-CHLORO, 2-METHYLPHENOXYACETIC ACID.
120
80
40
0
CONTROLS.
0.00001
0.0001
0.001
0.01
0.1
1.0
P.P.M. MCPA.

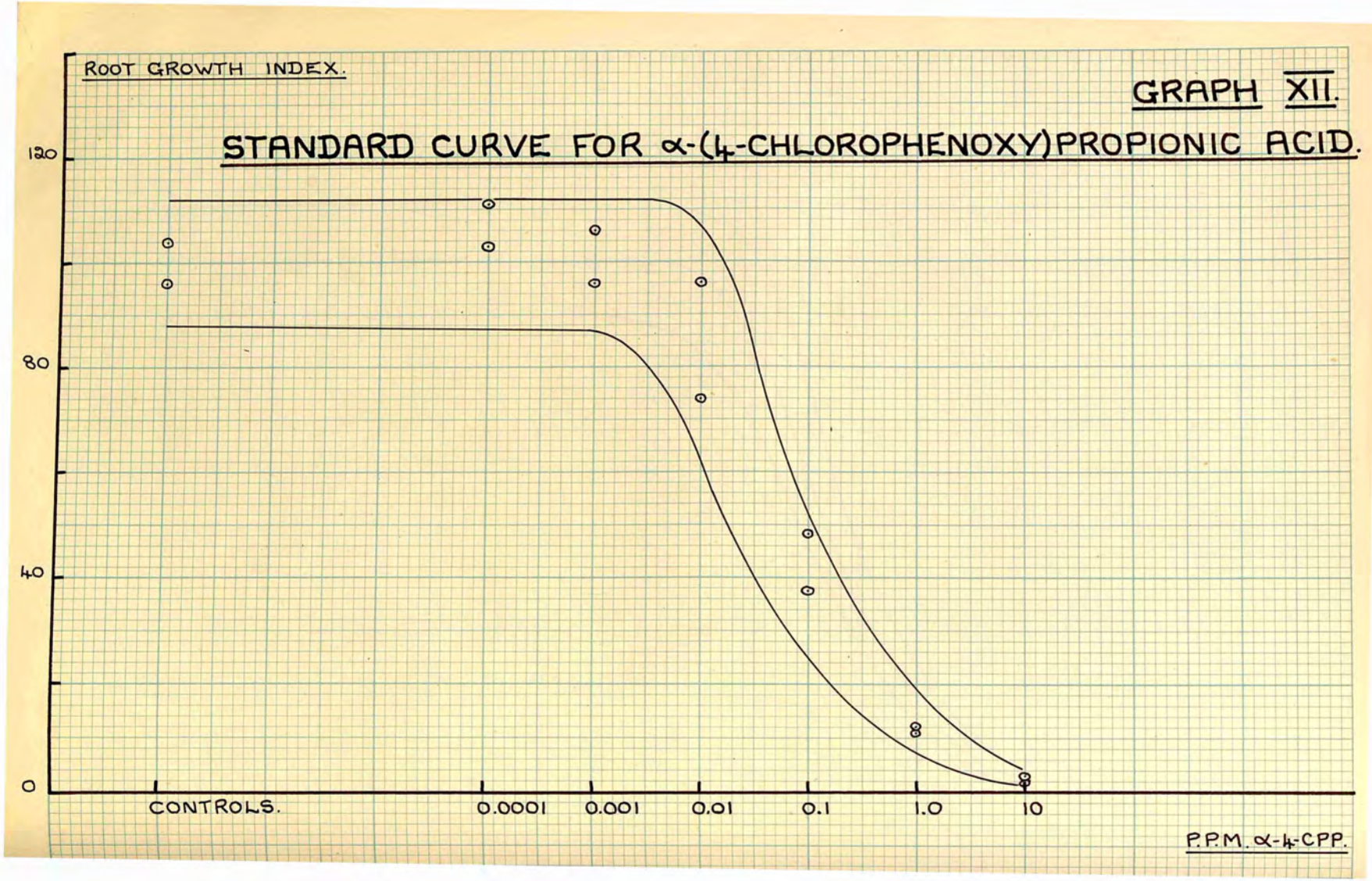
GRAPH XI.

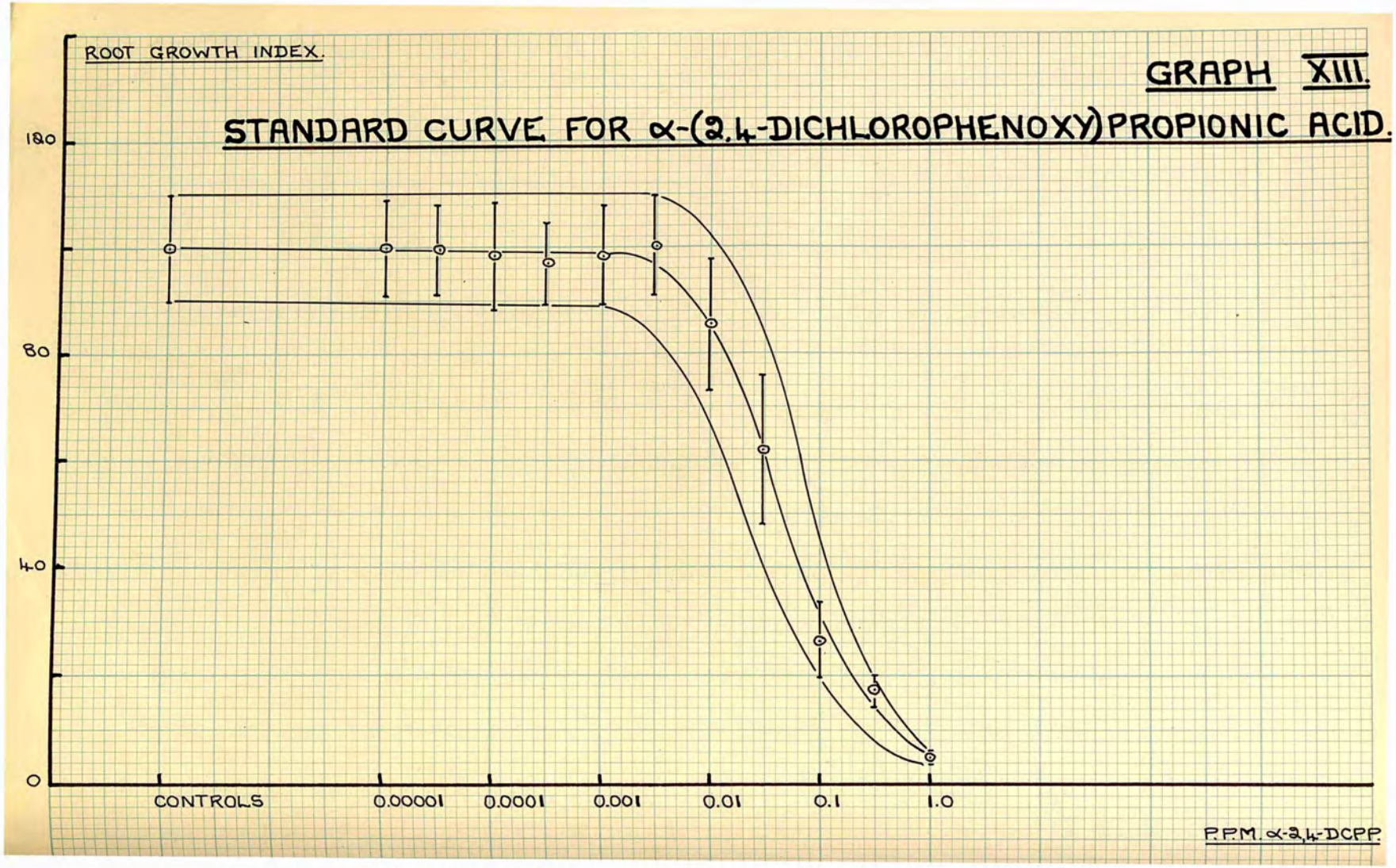
STANDARD CURVE FOR 2,4-DIMETHYLPHENOXYACETIC ACID.



GRAPH XII.

STANDARD CURVE FOR α-(4-CHLOROPHENOXY)PROPIONIC ACID.




ROOT GROWTH INDEX.
GRAPH XIII.
STANDARD CURVE FOR α-(2.4-DICHLOROPHENOXY)PROPIONIC ACID.
180
80
40
0
CONTROLS
0.00001
0.0001
0.001
0.01
0.1
1.0
P.P.M. α-2,4-DCPP.

concentration as standard so as to compare the relative inhibition produced by all compounds at that concentration. It is necessary to select a degree of inhibition and compare the different concentrations needed to produce it. It is difficult to determine the weakest concentration which will just cause complete inhibition. That concentration permitting 50% of control growth is determinable with a reasonable accuracy, for the R.G.I. / log. concentration curve is more or less linear in this region. Relative Toxicity may then be defined as the ratio of herbicide concentration permitting 50% of control growth to the concentration of a standard compound (eg. 2,4-D) also permitting 50% of control growth under the same test conditions and using the same test materials. Expressed as a percentage,

$$\text{Relative Toxicity} = \frac{\text{Conc. of 2,4-D permitting 50\% growth}}{\text{Conc. of other inhibitor permit. 50\% grow.}} \times 100$$

The test materials and conditions must be exactly the same for all compounds because different materials and tests give different relative values. The Cress Test was used throughout these experiments. While the values obtained for Relative Toxicity were of little direct interest, they did enable the compounds to be arranged in order of decreasing toxicity. This sequence was used for other comparative purposes.

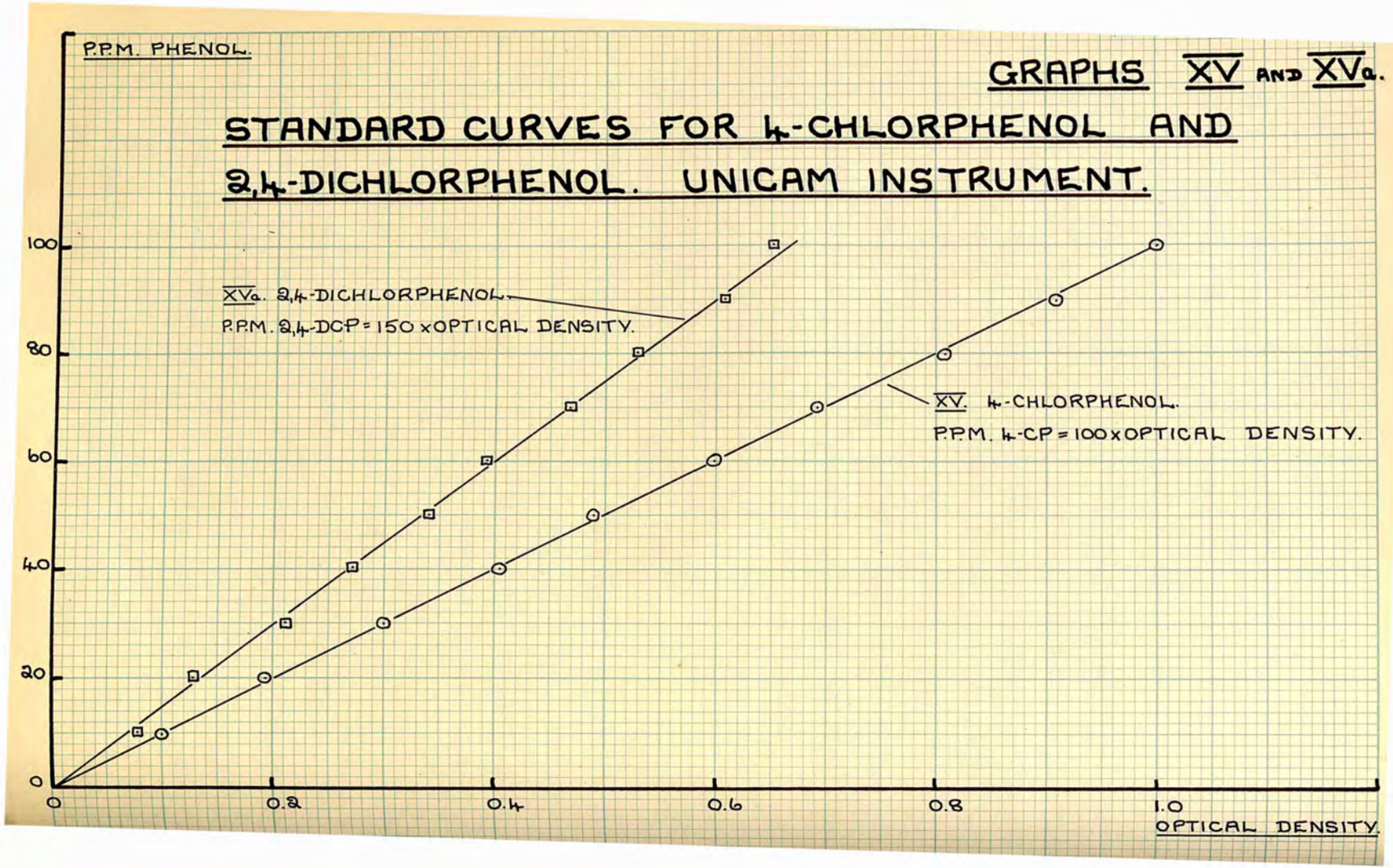
Colourimetric determination of phenols.

Use of the Porteus and Williams reagent 2,6-dichloroquinonechloroimide (99,) was precluded by the fact that two of the phenols to be determined, ie. 4-chloro and 2,4-dichlorophenol, were substituted in the para position and the method was not applicable to para substituted compounds. Attempts were made to use the method of Gottlieb and Marsh (38,) in which the phenol is coupled with 4-aminoantipyrene in the presence of alkaline ferricyanide. The method had been shown to work with samples of commercial 2,4-DCP but it gave very erratic results with perfusate samples containing soil extract. This may be attributed in part to difficulty in maintaining the pH at the optimum value which, according to the authors, is the most common source of trouble.

The method finally adopted and used throughout the research was an adaptation of that due originally to Folin and Ciocalteu (34,) and outlined in Snell and Snell (113,). A 1 ml. perfusate sample was run into 3 ml. of saturated sodium carbonate solution (almost 20% at 20°C). 1 ml. of Folin and Ciocalteu Reagent (supplied by British Drug Houses Ltd., Poole, Dorset.) was added followed by 5 mls. of distilled water, previously warmed to 30-35°C. The solutions were mixed thoroughly and allowed to stand for 20-30 mins. The intensity of the blue colouration produced was measured photometrically using either (a) an E.E.L. Colourimeter with a red filter or, (b) a Unicam Colourimeter set to a wavelength of 685 millimicrons. In each case the instrument was

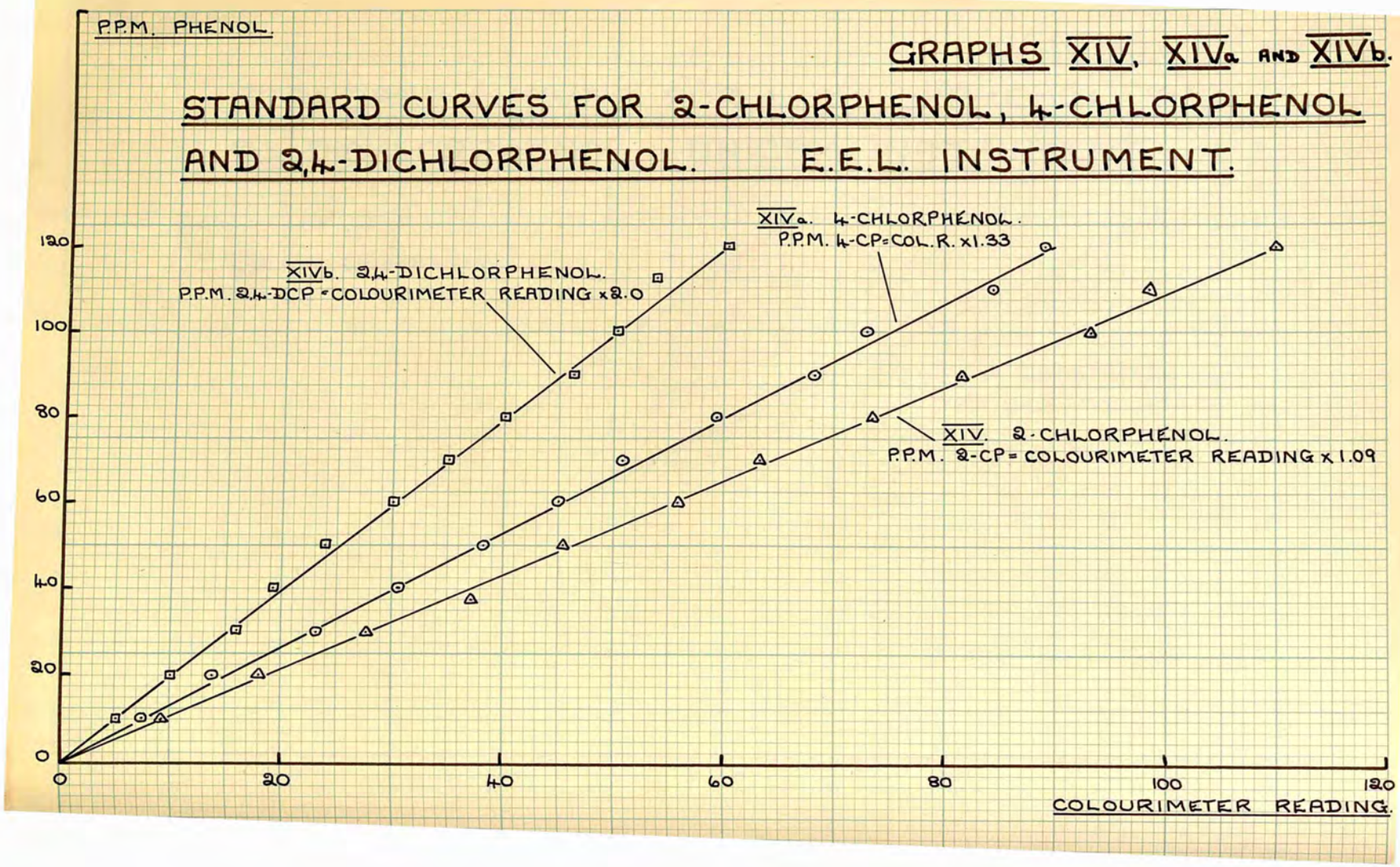
GRAPHS XV AND XVa.

STANDARD CURVES FOR 4-CHLORPHENOL AND 2,4-DICHLORPHENOL. UNICAM INSTRUMENT.



GRAPHS XIV, XIVa AND XIVb.

STANDARD CURVES FOR 2-CHLORPHENOL, 4-CHLORPHENOL AND 2,4-DICHLORPHENOL. E.E.L. INSTRUMENT.



preset to zero while containing a control cell in which the reaction had been carried through as above, but with one ml. of distilled water replacing the perfusate sample.
Standard Curves and factors relating colourimeter reading to phenol concentration were prepared for each phenol.

Standard phenol calibration curves.

A range of phenol concentrations from 1 to 100 ppm. was prepared by dilution from a 1,000 ppm. stock solution. 1 ml. portions of each concentration were treated as above and the colourimeter readings noted. Three determinations were made at each concentration and the mean value taken. These mean colourimeter readings were plotted against phenol concentration on a linear / linear scale. For each of the phenols tested, a linear relationship was found to hold over the range of concentrations used. Consequently, it was possible to calculate simple factors relating colourimeter reading to phenol concentration. Phenol concentrations higher than 100 ppm. were determined on diluted samples and allowance made for the dilution factor.
After the initial standing period of 20 to 30 mins., during which the blue colouration reached maximum intensity, very little further change occurred on standing, even for as long as 15 hrs.

Determination of iodide.

The study of halogen liberation during ring degradation of the halogenated phenoxyacetic acids is one method of following the degradation. Owing to the low,tolerated herbicide concentrations, it would probably have been impossible to detect liberated chloride against the background of natural chloride in the soil. Detection of iodide liberated from 4-iodophenoxyacetic acid seemed more likely to succeed. It was necessary (owing to the low expected concentration) to choose a micro-method for its determination. A bromination method, based on those reported in Milton and Waters (80,) was adopted.

It proved impossible to determine iodide directly in the perfusate samples. High and erratic values were obtained in preliminary tests. Even the stock solution of 4-iodophenoxy-acetic acid gave high "free iodide" figures so the erratic test results may have been due to interference by undecomposed acid in the perfusate. It is probable that iodide was liberated from the acid by bromine replacement during the test. It is possible that this difficulty could have been overcome by first extracting the unchanged acid from the perfusate sample. Time did not allow the elaboration of such a technique.

Measurement of Optical Activity.

At intervals during substituted α-phenoxypropionic acid perfusions, the perfusate was tested for possible development of optical activity. This would have indicated preferential decomposition of one of the enantiomorphs of the racemic mixture. No measureable optical activity was found. A Hilger-Spekker Polarimeter was used with a 2 decimetre tube water jacketed at 20°C. It had a sensitivity of 0.02° rotation using the mercury line. The low tolerated herbicide concentration and the low specific rotation of the separated isomers made the possibility of detection most unlikely unless a high degree of preferential breakdown had occured.

EXPERIMENTAL RESULTS.

I. Isolation and Study of Responsible Organisms.

(a). Introduction.

Several workers have attempted to isolate, and grow in pure culture, soil bacteria which they believed responsible for the destruction of herbicides in soil. Isolation usually proved easy but culture on synthetic media was difficult. Anderson and Baker (3,) found no evidence of 2,4-D breakdown in culture media , by micro-organisms isolated from treated soils. Newman and Thomas (89,) isolated, but did not identify, a number of species from active soils, which grew well on 2,4-D agar. These pure cultures could not decompose 2,4-D in liquid media but crude, mixed cultures did so slowly. The speed of breakdown could be increased by the addition of yeast extract. The highest concentrations used, 0.05 and 0.1%, were most effective. From their results they concluded that certain micro-nutrients, or a suplemental energy source, may be required by the pure cultures if they are to bring about 2,4-D breakdown. They also suggested that a toxic material is produced, during the breakdown, which inhibits the process. Audus (8, 9,) claimed to have isolated, on 2,4-D agar medium, an effective organism from enriched perfuser soil. He showed that a heavy suspension of these bacteria could break down 2,4-D when perfused over fresh soil or glass wool. The organism was identified as belonging to the common group of soil bacteria, Bacterium globiforme (20, 69,). Growth was maintained, through repeated subcultures, for up to 12 months on agar medium

in which 2,4-D was the sole carbon source. There was, however, a progressive loss of vigour accompanied by the appearance of pleomorphism. This was probably due to the unusual nature of the carbon source (21,). Growth in liquid media only occurred when traces of agar (0.1%) were added. It was suggested that the agar supplied an essential growth factor normally obtained from the soil. Preliminary experiments with soil extract tended to confirm this view.

Jensen and Petersen (56,) isolated, in pure culture, two active bacterial species from soil repeatedly treated with herbicide. The first, Flavobacterium aquatile, grew vigorously on normal bacteriological media and was found to be capable of decomposing 2,4-D. After repeated sub-culture it grew very feebly but could still break down 2,4-D when added to sterile soil. The second species grew much more feebly and, in description, closely resembled the Bacterium globiforme isolate of Audus. It could decompose both 2,4-D and MCPA. Its poor growth on media not containing either of these substances was said to indicate its being a mutant form, probably adapted to a few closely related cyclic compounds. Jensen and Petersen showed that these bacteria could decompose herbicide when added to fresh soil or when grown on solid media with herbicide as sole carbon source. On semi-solid agar media, or liquid soil-extract media, they could get no significant breakdown.

(b). Isolation of the effective organism.

The medium most commonly used for the initial isolation and subculture of believed active organisms, from perfuser enriched soil, consisted of :

Ammonium di-hydrogen phosphate.....0.1%
Potassium chloride.....0.02%
Magnesium sulphate, hydrated.....0.02%
Agar.....3%
Herbicide substrate..... 2,4-D or MCPA.....0.1%
or, 2,4-DCP.....0.02%
pH.....7.0

This pH was chosen for the medium as it agreed with that of the soil used and the perfusates. In a few instances 0.1% asparagine was added as an extra source of carbon and nitrogen. For comparative purposes, 2.5% Difco Nutrient Broth, with or without 1 % glucose, was sometimes used.

Isolation plates were spread on the appropriate medium, using drops of active perfusate which had been removed with a sterile platinum loop. They, and subsequent sub-cultures, were incubated at 25°C.

In some cases, especially on glucose broth or media containing asparagine, colonies appeared in a day or two. Usually 1 or 2 weeks were required before the numerous pin-point colonies appeared. Except on the enriched media, colony growth was very slow and they rarely exceeded 1 to

2mm. diameter, even after prolonged incubation. The colonies were opalescent, pale blue-grey to white and, in the early stages had a distinct iridescent sheen. Apart from contamination by sparsely growing, unidentified fungi (cf. Audus, 8, 9,), the primary isolates on herbicide media were, usually, remarkably pure and appeared to consist of practically only one type of colony and organism. Numerous examinations were made of the microscopic appearance, staining and fermentation reactions of pure cultures of these organisms. To obtain large virile cultures, for the fermentation reactions, single colony subcultures were first made from the isolation plate onto glucose broth slopes. The results of these examinations always agreed with the identification of the organism as belonging to the Bacterium globiforme group (13,).

The bacteria were usually coccoid or short rods on nutrient agar. On more austere media, or primary isolates (especially from older perfusers), many elongated forms were present, some showing clubbing or branching. Always non-motile. Staining proved to be very difficult, even with hot, strong carbol fuchsin. The difficulty seemed to be due to the ease with which stain was washed out of the cells. For this reason the common occurrence of Gram-negative staining was not beyond question. In some instances Gram-positive coccoid forms and Gram-positive granules were observed.

On 1% glucose agar a flat, smooth, soft, glistening growth occurred, with a translucent, iridescent sheen before it became too thick.

Contrary to the type character good growth was observed on broth.

In the presence of glucose, sucrose or lactose, nitrate, as sole nitrogen source, was reduced to nitrite. In the presence of mannose, growth and reducing action were absent. Glucose was readily utilised, sucrose and lactose less readily. In no case was there production of gas or detectable acidity. Potassium nitrate and ammonium di-hydrogen phosphate could both serve as nitrogen sources when glucose was also present. Asparagine and peptone could supply carbon and nitrogen.

As these fermentation reactions were not very selective and the morphology and staining reactions not very characteristic, it is possible that a number of closely related species, or slightly different biochemical mutants, may thus have been identified under the group heading of Bacterium globiforme.

What appeared to be the same species was isolated from perfusers which had been enriched to 4-CPA, 2,4-D, 2,4-DCP or MCPA when the inoculations were made onto the corresponding agar medium. Similar, but usually fewer, colonies were obtained when the isolation was made onto a medium containing either of the other substrates. Similar colonies occasionally arose, as contaminants, on 2,4-D or MCPA agar plates which had not been inoculated. It is therefore doubtful whether all the colonies arising on an inoculated plate were of truly adapted organisms, using the herbicide as substrate, or whether some were merely tolerant of the herbicide and were living on impurities in the agar.

(cf. Newman and Thomas, 89,). The fact that isolation on agar medium containing no organic substrate produced very few colonies, and that isolation onto herbicide media other than of the adapting compound produced fewer colonies than on media containing the adapting compound, suggests that most of the colonies did arise from adapted organisms. It also indicates that Bacterium globiforme plays a part in, at least, the initial stages of breakdown.

Chance contamination never showed on 2,4-DCP agar media and the number of colonies resulting from isolations was always small. This suggests that only organisms fully adapted to 2,4-DCP, or closely related compounds, could tolerate this highly toxic substance (22, 94,) and utilise it as a source of carbon and energy.

2,4-D / asparagine agar was more highly contaminated than 2,4-D agar alone. Growth of the B.globiforme type colonies was also better and they achieved a bigger final size. On glucose / nutrient broth agar contamination was at an even higher rate. Final colony size was also improved often reaching 5 mm., or more, in diameter. Fungal growth on this medium was so great that isolation of pure bacterial cultures was very difficult. While of little value for the isolation of effective organisms, the heterogeneous growth on glucose / broth agar did show that many soil organisms can tolerate fairly high herbicide concentrations, remaining viable and capable of rapid reproduction when supplied with a suitable carbon source.

In order to minimize risk of contamination and possible de-adaptation of active bacteria by more accessable substrates, subcultures were only made from isolates on agar media in which the adapting compound was the sole carbon source.

(c). Effects of subculture.

All attempts to obtain vigorous pure cultures of adapted organisms, growing on the adapting substrate in synthetic media, resulted in failure. When subcultures were made from single, well isolated, colonies onto agar slopes, containing the three inorganic salts (p52) and appropriate herbicide only, very slow growth was sometimes observed. Further subculture on the same medium produced no observable growth. Subculture from the isolation plate into a liquid medium of similar constitution nearly always resulted in complete failure. Rarely, a slight ring of growth occurred at the surface (cf. 9,) but this soon ceased and further subcultures failed.

If the subcultures were made onto media in which the herbicide was replaced by glucose or asparagine, or onto glucose / nutrient agar, vigorous growth resulted whether the media were liquid or solid. On glucose / nutrient agar iridescence appeared in one day and a thick growth in eight days.

Media containing 2,4-D as well as asparagine or glucose supported better growth than 2,4-D alone though the herbicide appeared to have a marked depressive action.

Judging by the rate of breakdown of herbicide by an enriched soil, the responsible organism(s) must have been very active so long as they were retained in the soil and perfusate and must also have been present in considerable numbers. They were capable of ready multiplication on media containing easily assimilable carbon though growth was markedly reduced, and eventually ceased,in the presence of herbicide. Many explanations of this anomalous behaviour were possible and experiments were carried out in attempting to find the true one.

(d). Possible reasons for subculture failure.

(i). Isolation shock.

In the soil,the adapted organisms may have had access to organic nitrogen supplies and / or readily available carbon other than the herbicide. The shock of transfer to a medium containing only inorganic nitrogen and herbicide carbon may have reduced their vigour to such an extent that adaptation to the new conditions was not possible and subcultures failed. Some support for this theory is provided by the positive results obtained on subculturing onto media containing organic nitrogen and / or available carbon (asparagine, glucose, nutrient broth,). Attempts were therefore made to transfer the bacteria in easy stages from a rich isolation medium to the more stringent three salts + 2,4-D medium. Peptone was found to be a good carbon and nitrogen source for the bacteria; a series of tubes of liquid media, of the following composition , was prepared

and sterilised at 15 lbs. pressure for 15 mins.

Tube.	Vol.of peptone water(2% + 1% sodium chloride)	Vol.of double strength "3 salt soln.	Vol.of 2,000 ppm. 2,4-D solution.	Sub-cultured on day.
1.	6 ml.	0 ml.	6 ml.	0.
2..	4 ..	2 ..	6 ..	10.
3.	2 ..	4 ..	6 ..	14.
4.	0 ..	6 ..	6 ..	18.
5.	0 ..	6 ..	6 ..	24.

Tubes (1) were each inoculated from separate young colonies isolated on a 1% glucose + 0.1% 2,4-D + "3 salts" agar plate, from an active 2,4-D perfuser. Some growth occurred in all tubes down to tube (4), the first to contain no peptone. Further subcultures into tubes (5) all failed, demonstrating again the inability of the organisms to survive on this austere synthetic medium.

As an extension of the above experiment, a series of media were prepared in which the peptone content was constant at 1% (0.5% NaCl) but in which the glucose content varied from 1 to 0% and the 2,4-D varied inversely from 0 to 0.1% (in one tube there was no glucose and 0.5% 2,4-D). Two sets of identical media were prepared and one set was solidified by adding 3% of agar. Two similar sets of media were prepared in which the glucose was replaced by mannose. In preparation of the media, according to the following table, the 2% sugar solution was either glucose or mannose. For the solid media, 6% agar was incorporated in the peptone water, by steaming, and the solution measured out while still fluid. The tubes of media were sterilised by autoclaving at 20 lbs.

for 20 mins. The solid media were sloped in the usual way.

Tube.	Vol. of 2% peptone + 1% NaCl water.	Vol. of 0.2% 2,4-D soln.	Vol. of 2% sugar soln.
1.	3.6 ml.	0.0 ml.	3.6 ml.
2.	3.6 ..	0.4 ..	3.2 ..
3.	3.6 ..	0.8 ..	2.8 ..
4.	3.6 ..	1.2 ..	2.4 ..
5.	3.6 ..	1.6 ..	2.0 ..
6.	3.6 ..	2.0 ..	1.6 ..
7.	3.6 ..	2.4 ..	1.2 ..
8.	3.6 ..	2.8 ..	0.8 ..
9.	3.6 ..	3.2 ..	0.4 ..
10.	3.6 ..	3.6 ..	0.0 ..
11.	3.6 ..	3.6 ml. of 1% 2,4-D giving a final conc. of 0.5%.	0.0 ..

The source of the inoculum for these series was a first subculture, onto glucose/3 salt agar medium, from a single colony isolated on 2,4-D/3 salt agar. The growth, typical of B. globiforme, was scraped off and suspended in 5 mls. of sterile peptone water. Single drops of the suspension were used to inoculate tubes (1) in each of the four series. Subculturing down each series was carried out whenever sufficient growth appeared to provide an inoculum for the next tube. Progress was as follows:-

i). Glucose-liquid media.

Tube.	1	2	3	4	5	6	7	8	9	10	11
Day of inoculation.	0	4	5	7	9	19	28	36	41	44	48

Growth was good as far as tube (8). Tube (11) was re-inoculated from (10) on day 53 but no growth was observed by day 62.

ii). Mannose liquid media.

Tube.	1	2	3	4	5	6	7	8	9	10	11
Day of inoculation.	0	4	5	7	9	19	28	36	41	44	

Growth was good as far as tube (8). No growth in tube (10).

iii). Glucose agar media.

Tube.	1	2	3	4	5	6	7	8	9	10	11
Day of inoculation.	0	4	5	7	9	19	28	36	41	44	53

Very good growth as far as tube (8).

iv). Mannose agar media.

Tube.	1	2	3	4	5	6	7	8	9	10	11
Day of inoculation.	0	4	5	7	9	19	28	36			

No growth on tube (8) and very poor on (6) and (7). Tubes (6), (7), (8) and (9) all re-inoculated from (5) on day 41. Still poor growth on (6) and (7), none on (8) and (9). All re-inoculated together with (10), from tube (5) on day 44.

With each sugar, growth was usually better on solid than in the corresponding liquid medium. With both solid and liquid glucose media, good growth was maintained down the series till the glucose concentration had fallen to about 0.2% (tube 8) and the ratio of glucose to 2,4-D was about 3:1. Beyond this point, growth in decreasing amount was maintained

up to tube (10), the first containing no glucose. There was no sign of growth following subculture into tubes (11) in which the glucose concentration was higher. Mannose media were less effective, the solid media failing to support reasonable growth after tube (5) which contained about 0.6% mannose and a ratio to 2,4-D of about 12.5 : 1. The liquid mannose media more closely resembled those containing glucose in supporting growth up to tube (8) though growth in (9) was poor and (10) failed completely.

Growth on mannose media, both solid and liquid, tended to become viscous.

Failure in these attempts to educate the organisms from very active cultures on rich media to growth on synthetic media suggests that the shock of isolation onto an unsuitable medium was probably not the reason for cultural failure. This is especially noteworthy as the time taken to traverse each series was longer than the time taken to enrich a perfuser to 2,4-D. Also, the increase in 2,4-D concentration was gradual whereas in a perfuser it was immediate.

(ii). Possible need of a supplementary carbon source.

In the previous experiment, growth was maintained down to a low glucose level but failed in the absence of glucose. This suggested that a supplementary carbon source might be required when the organisms were decomposing 2,4-D though, as peptone water had been found to support growth of B.globiforme, it is difficult to explain why growth should

have ceased in the absence of glucose, with peptone still present. It must be assumed that,in some way, 2,4-D inhibited peptone utilization by the organisms. This possibility will be discussed later (71 and 201,).
The supplementary carbon source might be assumed to function by supplying energy to be used at some stage of the 2,4-D degradation, or by supplying essential molecular fragments not resulting from 2,4-D breakdown. These latter might be needed for cell maintainance and reproduction or as co-reactors in the degradation process. Newman and Thomas (89,) also suggested the possible requirement of a supplemental energy source.
Glucose seemed able to fulfil the requirements, so a series of media were prepared in which the 2,4-D concentration remained constant but the glucose content varied over a wide range. The wide range was chosen because too high a glucose concentration might have resulted in de-adaptation of the bacteria, or a preferential utilization of the sugar, while too low a concentration might not have fulfilled the requirements of the system.
A 10% glucose solution was prepared in neutral, double strength, "three salt" solution. From this stock solution, dilutions were prepared, in the same salt solution, to contain 1.0, 0.1, 0.01, 0.001, 0.0001, 0.00001, and 0.000001% of glucose. Eight test tubes were set up, each containing 5 ml. of 0.1% 2,4-D and 5 ml. of one of the glucose solutions. In this way each tube contained 10 ml. of normal strength "three salt" solution with 2,4-D at 0.05% and glucose

concentration of 5% down to 0.0000005%. The ratio of glucose to 2,4-D varied from 100:1 down to 1:100,000. One tube was prepared to contain 0.05% of 2,4-D only. The tubes were plugged and sterilised at 15 lb. pressure for 15 mins. The solution containing 5% glucose showed some browning. The others were not obviously affected. To prepare the inocula, 1 ml. of sterile, single strength "three salt" solution was mixed with 1 ml. of a five day old bacterial growth in a solution containing approximately 800 ppm. 2,4-D and 0.2% glucose in 1% peptone water (tube (8) of the glucose/2,4-D liquid series in the previous section). Five drops of inoculum was added to each tube. After thorough mixing, 1 ml. samples were removed aseptically from each tube. The samples were sterilised and assayed as usual. The tubes were incubated at 25°C, aeration being achieved by shaking at frequent intervals. After 20 days there was still no evidence of 2,4-D breakdown in any tube. Sampling and assaying were discontinued. All tubes showed an opalescence indicative of slight growth during the first two or three days. Only in the 5% glucose medium did growth increase and a ring of growth formed at the surface.

Assuming the inocula used in this experiment were derived from cells which, in the soil, had been capable of decomposing 2,4-D, it would appear that either a) they had lost this power during the isolation, etc. processes or, b) that provision of a supplementary carbon source, in the form of glucose did not fulfil the requirements of the organisms or, c) that the experiment was not continued long enough. This

latter explanation is unlikely for the normal adaptation time of fresh soil to 500 ppm. 2,4-D would be no more than 20 days.

(iii). Micronutrients.

1). Introduction.

The slow, but definite growth on the isolation plate and rapid loss of vigour on subculturing onto herbicide media, but not on richer media, suggested that the bacteria might be able to synthesise an essential micro-nutrient if given glucose, or some other "easy" carbon source, but not from 2,4-D breakdown products. Alternatively, the micro-nutrient, organic or inorganic, might only be essential when an unusual compound such as 2,4-D was the sole carbon source. If the micro-nutrient hypothesis is true, it must be assumed that the drop of liquid used for the initial inoculation must have contained sufficient micro-nutrient to permit the observed slight growth. Further carry-over to the subculture medium would be too small to sustain further growth. The fact that primary subcultures were sometimes successful on agar media, but very rarely in liquid media, suggested that agar might contain a small amount of the micro-nutrient, sufficient to maintain growth if supplemented by a carry over yet probably too low to be active itself. Audus (8, 9,) had already suggested that agar might contain such a substance, while Newman and Thomas (89,) had concluded that "growth factors were probably required".

A number of micro-nutrients were tried for growth promoting activity in 2,4-D synthetic media using organisms isolated from various enriched perfusers.

(iii). 2). Soil extracts.

The 2,4-D decomposing organisms must derive some benefit from existence in soil which is not available on "three salt" agar. Soil extract seemed a likely source of stimulant for these cultures (see Audus, 9,). Several different types of extract were prepared from air-dried, sieved (2-4 mm.) soil as used in the perfusers. The extracts were used in compounding media both for initial isolation plates and for subculturing purposes.

Neutral aqueous extract.

200 gm. of soil was shaken at intervals during incubation for several days at 25°C, with 500ml. of glass-distilled water. The liquid was filtered off and found to be very nearly neutral. 3% agar/"three salts"/0.1% 2,4-D medium was prepared from the filtrate and autoclaved for 15 mins. at 20 lbs.

Acid soil extract.

500 gm. was extracted with 500 ml. of 0.05N hydrochloric acid at 20 lbs. pressure for 20 mins. then filtered. 3% agar/"three salts" medium was prepared by steaming with the filtrate, neutralised with N/1 sodium hydroxide and filtered hot. 2,4-D was added to a concentration of 0.01% and the medium tubed and sterilised at 15 lbs for 20 mins.

Acid soil extract + full range of nutrient minerals.

200 gm. of soil and 200 ml. of 0.05N hydrochloric acid were autoclaved together for 20 mins. at 20 lbs. The suspension was filtered hot and the filtrate, made slightly ammoniacal, autoclaved again for 20 mins. at 20 lbs. After further filtering, 2,4-D was added to give 0.1%, the appropriate amounts of nutrient salts (in solution) to make the medium of Nutman et al (92,) and a full range of accessory elements (52,). The pH was adjusted to 7.0, half of the liquid was stiffened with 3% agar and both types of media were tubed and autoclaved for 25 mins. at 15 lbs.

(iii). 3). Ammonia precipitate from acid soil extract.

1,000 gm. of soil was extracted with 1,000 ml. of 0.05N HCl at 20 lbs. for 20 mins., then filtered. The 600 ml. of filtrate was diluted to 750 ml. and neutralised with concentrated ammonia solution. No precipitate formed. The solution was halved and excess ammonia added to one half. A chocolate-brown flocculent precipitate resulted. Excess ammonia was boiled off and the precipitate collected on a tared filter paper. Filtrate and precipitate were autoclaved for 20 mins. at 20 lbs. Approximately 3.5 gm. of partially dried precipitate was obtained. It was scraped from the paper and digested with 1 ml. of hot, concentrated, "Analar" hydrochloric acid, in which it was only partially soluble. After neutralising with concentrated ammonia, 1.5 gm. of agar and 5 ml. of 1% 2,4-D solution was added, and the solution made up to 50 ml. with distilled water. The filtrate from the above precipitate and the other half of the original

extract were similarly made up to 0.1% 2,4-D and 3% agar. All three types were tubed and autoclaved at 20 lbs. for 15 mins.

All the types of soil extract media, prepared as above, were tried as isolation or subculture media for several distinct isolations. On the whole, there was no evidence of stimulated growth above thaton similar media not containing extract. In some cases the medium prepared from the ammonia precipitate showed slight stimulatory activity but this was not a consistent phenomenon. Some of the old cultures isolated by Audus (8,) failed to revive on these soil extract media, though they did when subcultured onto nutrient glucose agar. The almost total failure of these soil extracts to stimulate growth on 2,4-D agar suggests that either a). the soil and/or extracts do not contain the hypothetical growth factor or factors, or b). that if originally present, destruction occurred during the extraction or sterilisation processes. As a wide range of trace elements was included in one of the extracts,it is a safe conclusion that the factor(s) is not mineral in character. It is possible that, in the extracts, the factor(s) may have been in too low a concentration to be effective. It may have been concentrated either by direct precipitation or by absorbtion onto the ammonia precipitate. This might account for the occasional appearance of stimulation on media prepared from the precipitate.

(4).Expended perfusate as a stimulant.

Early in the present century, Wildiers showed that yeast cells did not survive transfer to fresh sugar solution unless a large inoculum was used. This effect was shown to be due to certain essential micro-nutrients,in the liquid of the inoculum, which had been produced by the yeasts themselves from the sugar. In a like manner it was thought possible that the 2,4-D organism might be producing more than its requirements of growth factor(s) while breaking down 2,4-D in the soil. The amount carried over into the isolation medium may not have been sufficient to allow vigorous, self-satisfying growth to start. Any excess of the factor(s) in the old perfusate might be capable of stimulating growth on media into which it was incorporated. As spent perfusate was also an aqueous soil extract it had a double chance of being stimulatory.

The perfusate was drained from an active perfuser, which had contained 250 ml. of 0.1% 2,4-D , as soon as the 2,4-D content dropped to a non-detectable level. It was filtered free from soil particles and used in the preparation of various media. A cold aqueous soil extract was prepared (50 gm. in 250 ml.) in order to prepare comparison media for distinguishing between possible perfusate effects and those due to soil extract alone. Double strength "three salt" medium was prepared from each liquid and the following media from these solutions: a) glucose+perfusate, b) glucose+soil extract, c) mannose+perfusate, d) mannose +soil extract.

Tube.	Volume of perfusate	Volume of 2%glucose	Volume of 0.2%2,4-D	Inoculated. (Day)
1.	5 ml.	5 ml.	0 ml.	0.
2.	5 ml.	2.5 ml.	2.5 ml.	3.
3.	5 ml.	1.25 ml.	3.75 ml.	5.
4.	5 ml.	0 ml.	5 ml.	7.
5.	5 ml.	0 ml.	5 ml. of 1%.	

Fair growth occurred in all tubes (1). Growth continued but at a slower rate in tubes (2). There was very slight growth in tubes (3), being better on glucose than on mannose. There was no growth in tubes (4).

Tubes of other sugar media were prepared in the same way, each containing 5 ml. of soil extract or perfusate, 2.5 ml. of 0.2% 2,4-D and 2.5 ml. of 2% sugar solution,(sucrose or lactose).

A bacterial suspension was prepared by dispersing a single isolated colony, from a primary isolation plate, in 1 ml. of sterile "three salt" medium. Single drops were used to inoculate the sucrose and lactose media and all tubes (1) of the other media. In these series, further subcultures were made on the days indicated in the above table.

Fair growth occurred in all media 0.5% or more of sugar and no growth when sugar was absent. There was little variation between sugars. Growth was no better than could be expected in purely synthetic sugar/2,4-D/"three salt" media.

Plates containing 2,4-D/"three salts"/spent perfusate/agar

were inoculated directly from active perfusers. The characters of the isolates did not differ from those normally obtained on media not containing spent perfusate.

From these experiments it would appear that self stimulatory products are not formed by the bacteria acting on 2,4-D.

5). Effect of calcium ions.

Taylor (122,) found that calcium ions were the factor in soil extract which could overcome the inhibition of cultures of some soil bacteria when grown on media containing tryptone. The amount of calcium required was positively correlated with the tryptone content of the medium and he concluded that the calcium was acting, not as a simple nutrient but, as an antagonist of some inhibitor in the tryptone. The description of Bacterium globiforme agrees with that of some of the soil organisms used by Taylor. It was decided to test the effect of calcium ions on B.globiforme cultures even though calcium salts and soil extract had been included in previously tested media.

Taylor found 20 to 100 ppm. of Ca^{++} to be quite effective so 40 ppm. of calcium,as nitrate, was incorporated into normal "three salt" agar containing 0.1% of either 2,4-D, 2,4-DCP, MCPA or asparagine. In some instances the medium contained 0.1% of both asparagine and 2,4-D. Inoculations were made directly from perfusers enriched to the corresponding herbicide, or to 2,4-D in the

case of asparagine media.

Only in the case of 2,4-D medium (alone) was there some evidence of the calcium ion being beneficial. On all the other media growth appeared to be no better, or even worse, in the presence of calcium.The use of Ca^{++} along with other micro-nutrients is mentioned later.

Though outside the strict limits of the present work, some of the results achieved and some results published by other workers lead to the conclusion that there may be a connection between the metabolism of some micro-organisms, calcium ions, tryptophane (or other peptone constituent) and plant growth substances. The following observations have been made by other workers:-

a). Taylor (122,) showed that calcium ions could overcome the inhibitory action of some substance in tryptone medium.

b). Kries (62,) and others have shown that lime can cause the inhibition of herbicide breakdown in soil, though this may have been a pH effect rather than a calcium ion effect.

c). Dubos (29,) found that peptone, or tryptophane, can overcome hormone inhibition of some bacteria.

d). From the limited number of experiments in the present research, it appears that 2,4-D can inhibit the utilization of peptone by B.globiforme (see p.61.) and that calcium ions may inhibit the utilisation of 2,4-D by the same species.

Other evidence will be presented later together with

a discussion of the theoretical implications (p.201).

(iii).(6). Effect of yeast extract.

Newman and Thomas (89,) found that in a synthetic medium containing 500ppm. 2,4-D they sometimes got complete decomposition in less than 21 days, providing mixed cultures were used and 0.05 to 0.1% of yeast extract was added to the medium. In the absence of yeast extract, or using it at lower concentrations, no decomposition occurred in 42 days.

In the present experiments, a range of yeast extract concentrations was added to 2,4-D solution in the usual "three salt" medium. 2,4-D was at 0.1% and yeast from 0.1 down to 0.000005% with controls containing no yeast. The solutions were placed in tubes plugged with cotton-wool which also supported central bubbler tubes through which sterile air could be blown. They were sterilised by steaming for half an hour on each of three successive days. A tube containing 2,4-D, but no yeast, was inoculated from a single B.globiforme colony on a 2,4-D agar plate and after 10 days all the other tubes were inoculated from it, adding 1 ml. to each. Alternate tubes were sampled for assay at the start of the experiment and after a further 6 and 50 days. There was no decrease in the apparent 2,4-D content of each tube. Seven days after inoculation, bacterial counts were made in those tubes containing 0.1, 0.01 and 0.001% yeast extract. Counts of approximately 240,000, 64,000 and 4,000 per cub.mm. were obtained, respectively. A tube containing 0.1% asparagine

in the same basic "three salt" medium, which had been similarly inoculated, gave a count of approximately 160,000 per cubic millimetre. Forty eight days after inoculation the same cultures were again counted. There was a marked drop in bacterial numbers to 37,500, and 5,000 for the two higher yeast concentrations respectively and approximately 40,000 per cu.mm. in the 0.1% asparagine.

In those tubes containing the higher yeast concentrations good growth had occurred, as judged by opalescence, though there was no evidence of 2,4-D breakdown. This suggests that the organisms were not using the extract as a source of vitamins but as a carbon source to the exclusion of 2,4-D. The direct relationship between bacterial count and yeast extract concentration lends further support to this theory. The marked decrease in numbers by the second count could be due to autolysis or phage activity. Complete disappearance of colonies from agar slope cultures after prolonged incubation, even on favourable media, had often been observed and tends to support the phage theory.

(iii). (7). Effect of vitamin B_{12}.

Lochead and Thexton (70,) showed that soil extract was capable of stimulating the growth of some micro-organisms, in pure culture, which were unaffected by yeast extract.They found that in some cases the soil extract could be replaced by vitamin B_{12} at a concentration of 2γ per ml. Only a limited amount of

vitamin B_{12} was available at the time of the present experiments and it was therefore impossible to carry out a comprehensive series of tests. Some tests of the vitamin were made in conjunction with yeast extract and calcium ions. 20 ml. of 0.1% 2,4-d in normal "three salts" medium was pipetted into each of six tubes and calcium nitrate added to some of them to give a concentration of 50 ppm. of the calcium ion. All six tubes were plugged and autoclaved for 20 mins. at 15 lbs. Calcium phosphate did not precipitate. Steam sterilised yeast extract and vitamin B_{12} from a sterile ampoule were added aseptically to appropriate tubes. The following series was produced:-

(a). 20 ml. of basic medium + 10γ vit.B_{12} (ie. 0.5γ per ml.)

(b). 20 ml. of basic medium containing 50 ppm. Ca^{++}.

(c). 20 ml. of basic medium + 0.025ml. yeast extract (ie.500 ppm.).

(d). 20 ml. of basic medium containing 50 ppm. Ca^{++} and 500 ppm. yeast extract.

(e). 20 ml. of basic medium containing 50 ppm. Ca^{++}, 500 ppm. yeast extract and 10γ vitamin B_{12} (0.5γ per ml.).

(f). 20 ml. of basic medium only.

A single colony subculture, originally isolated from a 2,4-D enriched perfuser, was suspended in sterile water and equal amounts added aseptically to each of the above tubes.They were incubated at 25°C. 1 ml. assay samples were taken from tubes (5) and (6) at the beginning of the experiment and after 10 and 62 days.

Clearly visible growth occurred only in those suspensions

containing yeast extract which was, presumeably, again acting as a readily available carbon source for there was no detectable 2,4-D breakdown in tubes (5) and (6).
While it did not appear to be effective, vitamin B_{12} cannot be ruled out as a growth promoter owing to the limited scope of the experiment.

(iii). (8). Effect of menaphthone (2-methyl,1:4-naphthoquinone). Hey and Hope (49,) suggested that the susceptibility of plants to herbicidal hormones was related to their vitamin K content, the higher the vitamin K content the higher the susceptibility. They considered the vitamin to be a co-reactor when the hormones are used as growth regulators. Assuming this suggestion to be correct, it was thought that vitamin K might also play a part in the breakdown of 2,4-D by soil micro-organisms, especially as vitamin K is known to be produced by certain micro-organisms. Menaphthone (vitamin K_3, 2-methyl,1:4-naphthoquinone) was chosen for the experiments as it was more readily available than the true vitamin which it can usually replace, either in its own right or as a precursor. Six types of media were produced each with 3% agar, the usual "three salts" and,in addition, 1 ml. per litre of the A-Z trace element solution. Some of the media contained 0.1% 2,4-D and all were autoclaved for 5mins. at 20 lbs. Steam sterilised Menaphthone solution was added aseptically to some of the media to give the following final media:-

(a). Basic medium only, nothing added.

(b). Basic medium + 5 ppm. Menaphthone.

(c). Basic medium + 1 ppm. Menaphthone.

(d). Basic medium + 0.1% 2,4-D only.

(e). Basic medium + 0.1% 2,4-D and 5 ppm. Menaphthone.

(f). Basic medium + 0.1% 2,4-D and 1 ppm. Menaphthone.

The inoculum was prepared by emulsifying a single colony, from a 2,4-D primary isolation plate, in 0.5 ml. of sterile water and spreading single drops of this on each of the six slopes. The tubes were incubated at 25°C.

Growth was poor on all media but appeared to be best on 2,4-D alone (type d,) and better on Menaphthone alone (types b and c,) than on mixtures containing 2,4-D and Menaphthone. Growth was better in the presence of 1 ppm. rather than 5 ppm. Menaphthone either alone or with 2,4-D. Menaphthone appeared to be a bacterial growth inhibitor, at the concentrations used, and to add its inhibition to that of the 2,4-D. Lower Menaphthone concentrations were not tried.

(iii).(9). Dialysed agar media.

From the range of substances tested it seemed unlikely that lack of a micro-nutrient was the reason for failure of the 2,4-D organism to grow in pure culture and break down 2,4-D. Alternatively, the viability of the organisms could have been reduced by repeated subculture if an inhibitor was present in the agar. In the simplest case the inhibitor would need to be water-soluble.

An attempt was made to "purify" the agar by dialysis. A quantity of agar was placed in a "Cellophane" bag and dialysed against running water for 10 days. The bag was kneaded at frequent intervals so as to ensure treatment of the entire contents. The dialysed agar (approximately 2.5% on a dry weight basis) was used to prepare two batches of media. Each contained the normal concentrations of the "three salts" and "A to Z" trace element solution. 0.1% 2,4-D was incorporated into one batch only. Two similar batches of media, as controls, were prepared from undialysed agar. A number of tubes of each type of medium were inoculated, using single drops of perfusate from a 2,4-D enriched perfuser.

Even after prolonged incubation only slight growth was evident on each of the four types of media. Those containing 2,4-D were no better than those without, suggesting that no breakdown was occurring. If anything, growth was slightly better on undialysed agar, suggesting that a water soluble toxin is not present and that undialysed agar may contain a small amount of utilizeable organic matter which can support growth of the organisms at a slow rate.

Conclusions from work on pure cultures.

As a result of the range of experiments reported above it seems safe to conclude that the inability of B.globiforme to grow well on 2,4-D synthetic media, and produce evidence of 2,4-D breakdown, was not due to lack of suitable nutrients,(macro or micro) nor to the presence of a water soluble toxin, in the media employed. It must be admitted that if any essential micro-nutrient, as in soil extract or "spent perfusate", were thermolabile it may have been destroyed during the processing of the media. An experiment described later (p.127) will show that a thermolabile factor was responsible for the ability to transfer adaptation to MCPA from an enriched perfuser to fresh soil, using the perfusate. Boiled perfusate was inactive. The presence of a thermolabile stimulatory substance cannot be ruled out though it is more likely that adapted organisms were the responsible factor. The conclusive experiment, using filter sterilised perfusate, was not carried out.

As the micro-nutrient theory seems untenable some other explanation is required of the inability to obtain 2,4-D breakdown in synthetic media. After the initial lag phase, adaptation (in a perfuser soil) was a very rapid process and, judging by the relatively rapid rate of breakdown of subsequently added 2,4-D and some other compounds, each perfuser must have contained a large number of actively metabolising bacteria. On the other hand, the rate of growth on the primary isolation plate was always extremely slow and

the number of colonies appearing was always much smaller than might be expected. From these observations it was thought that the main mass of active bacteria might be in the perfuser soil and that the few in, and isolatable from, the perfusate may have been atypical of the population. The results of one experiment did suggest that such a differential distribution may have existed though, as adaptation to two substrates was involved, the results were not entirely unambiguous. A perfuser had been enriched first to 2,4-D and then to 2,4-DCP. A small amount of the active soil was taken from well below the surface of the column and shaken with about twice its volume of sterile, glass-distilled water. After allowing it to settle, the clear, supernatant liquid was used to inoculate three different types of "three salt" + organic substrate + agar media:-

a). 1% "Difco" glucose broth.

b). 200 ppm. 2,4-DCP.

c). 1,000 ppm. 2,4-D.

A similar series of media were inoculated with perfusate from the same perfuser. All six lots were incubated for 10 days at 28°C. with the following results:-

	Perfusate as inoculum.	Soil susp. as inoculum.
Glucose broth agar.	+++++	+++++
2,4-DCP agar.	+	++++
2,4-D agar.	++	+++

In each case the colonies and organisms were of the B.globiforme type only. As indicated in the above table, the soil appeared to be a better source of organisms than did the

perfusate. Those from the soil seemed much more able to utilize 2,4-DCBIt had been usual to obtain mixed cultures on glucose media, even from 2,4-D enriched perfusers of long standing, and the apparently pure growths obtained in this experiment may have resulted from selective toxic action of 2,4-DCP towards all organisms not concerned in its breakdown. It had been noted previously that there were less contaminants in isolates from 2,4-DCP perfusers, or onto 2,4-DCP agar, than with the 2,4-D analogues.

It had been found that when spent perfusate from an enriched perfuser was fortified with new substrate and then perfused over fresh soil, breakdown occurred in a shorter time than the normal lag for the compound in question. This was observed with all phenoxy-acids which could be broken down in the soil but attempts to transfer 2,4-DCP adaptation in the same way always failed. The results of the experiment outlined above may give a partial explanation of the anomaly. Ability to shorten the lag phase proved of great practical value for the production of enriched perfusers. Though carried out many times, with several different compounds, it only rarely happened that the lag phase was completely eliminated. If it is assumed that the organisms in the perfusate are typical of the total active population and that only one species of bacterium (B.globiforme?) is responsible for all the stages in the complete breakdown of 2,4-D, etc., then it is difficult to explain satisfactorily the "lag-phase shortening" phenomena. It may be argued that, in the fresh lot of soil used, a certain amount of more readily available organic substrate

(or an inhibitor) was present and that the added active organisms could not proceed with the herbicide breakdown till this other substance had been eliminated. In this way the normal lag phase could be regarded as the sum of elimination time + true lag period for the compound while the transferred adaptation lag would be elimination time only. This is an unlikely explanation for, though the normal lags for different compounds might be expected to be different due to variations in the adaptation time component, all compounds should have had the same transferred adaptation lag dependant only on elimination time.

If it is postulated that the active perfusate contained only very few adapted organisms, then they may have remained quiescent for a while in the fresh soil before erupting into a rapid reproductive and metabolic phase marking the end of the transferred adaptation lag period. Alternatively, division and metabolism of these cells may have constituted an auto-catalytic system in which the cell numbers increased exponentially. Though herbicide breakdown may have been taking place from the start, the rate for some time would be too low to be detectable by the assay procedure. Eventually the active cell population would pass a certain critical size and breakdown would be at a detectable rate, reaching a steady value when the active population inevitably reached an equilibrium size.

It is hardly likely that the transferred adaptation lag was caused by the sudden increase in herbicide concentration suppressing the metabolism of the active bacteria. Such a

lag did not occur when the concentration in an enriched perfuser was suddenly increased by filling.

In the primary enriched perfuser it is possible that the adapted organisms were metabolically active but reproducing only very slowly and in equilibrium with the mortality rate. When a proportion of these organisms was transferred to fresh soil (in the perfusate) their reproductive activity may, after a delay, have been temporarily restored. The restored activity may have been due to the presence of an essential factor in the fresh soil and the reproductive rate may have returned to its equilibrium value when this factor was depleted to a limiting level.

Division of the bacterial cells may have been inhibited by moderate herbicide concentrations without affecting their metabolism (pure cultures on 2,4-D media grew very slowly) Higher concentrations appeared to be toxic, (see section on 2,4-D perfusions, p.108) this may have been a result of the reproductive rate being lowered below the existing mortality rate (Poole and Hinshelwood, 98, found a similar effect of phenol on Bacterium lactis aerogenes). A drop in the active population size to a lower equilibrium value would be the inevitable outcome. As the herbicide concentration in a perfuser was slowly lowered by bacterial action, cell division would slowly increase and further accelerate the breakdown. This may have constituted the auto-catalytic system referred to previously (p.81). Adding new herbicide to an enriched perfuser did not create a new lag phase

because a large active population would already be present and breakdown would soon reduce the herbicide concentration below the inhibitive level, allowing the active population to maintain a fairly constant size. Sudden increase in herbicide concentration around the few organisms in spent perfusate would lower their reproductive rate to a point where only slow build up in active population size could occur. Breakdown rate for some time would be very slow giving the appearance of a new lag phase, the transferred adaptation lag.

Several workers (7, 23, 62, 89, 90, 97, 123,) have produced evidence suggesting that the intermediate breakdown products of 2,4-D have a stimulatory effect on higher plants and there is some evidence that bacteria may also be stimulated.(57, 58, 76, 138,). If it is assumed that these breakdown products do stimulate bacteria, then another explanation of the transferred adaptation lag is possible. Fortification of the perfusate before transferring it to fresh soil may inhibit division of the relatively few active cells present. Slow breakdown will remove the herbicide (inhibiting?) with the simultaneous formation of stimulatory breakdown products. Cell division and total metabolism would again follow an exponential (auto-catalytic) curve, resulting in termination of the lag phase and, eventually, complete breakdown of the herbicide. The negative effect of spent perfusate added to 2,4-D culture media tends to undermine this theory of stimulatory breakdown products though it is possible that the breakdown had progressed beyond the point

of stimulation or that the active substance was thermolabile and destroyed during sterilization of the media. The possible existence of a thermolabile agent has already been pointed out (p.78).

Though the theories outlined above go some way towards explaining the normal and transferred adaptation lag phase phenomena, they do not explain the failure of pure cultures to grow and break down herbicides in synthetic media. Each of the factors discussed above may have been partly responsible though some form of symbiosis seems to lend itself to better explanations. In perfusers the various active species, or strains,might not be uniformly distributed between perfusate and soil. The soil might contain all types participating in the breakdown chain but the perfusate might contain few organisms and they capable of carrying out only one phase, probably the first, of the chain of reactions. Newman and Thomas (89,) found that they could obtain a rapid breakdown of 2,4-D by using mixed cultures of unidentified organisms, isolated from pretreated soil. Pure cultures of the same orga organisms were ineffective. With this in mind, a number of experiments was carried out using what amounted to mixed cultures prepared from active perfusates or active soil suspensions.

Experiments with crude, mixed cultures.

These experiments followed on after normal perfusions and the results will therefore be presented in more detail in the appropriate sections dealing with perfusion of 4-CPA, 2,4-D and MCPA. An outline only of the results will be presented here.

Two sources of active, crude, mixed cultures were used, a). perfusate drained from enriched perfusers and paper filtered to remove soil and other suspended matter, b). the clear liquid obtained after shaking enriched perfuser soil with sterile water and allowing it to settle. In each case, 250 ml. of liquid was used with herbicide added to a concentration of 100 ppm. Sometimes, appropriate amounts of the three usual mineral salts were added though these had no apparent effect. The liquids so prepared were either, a). perfused in sterile apparatus over sterile, acid washed, broken pot, or b). incubated with similar pot or, c). were incubated alone at 25°C.

Both 4-CPA and 2,4-D could be decomposed in these ways, though the breakdown rates were very slow when compared with a normal active perfuser. This was especially true in the case of 2,4-D. It was found possible to further transfer the 2,4-D decomposing activity by adding 50 ml. of one of these active mixed cultures to 200 ml. of fresh, sterile 2,4-D solution. 4-CPA active suspensions retained their vitality through several additions of herbicide concentrate.

The low rate of breakdown by mixed cultures, relative to that

of a perfuser, may have been due to a number of causes such as:-

a). absence, or sub-optimal concentration, of an essential nutrient (micro or macro) in the synthetic media. From previous evidence this is unlikely.

b). more than one type of organism may co-operate in the breakdown. The mixed cultures may not have contained them in the optimum relative proportions.

c). the large surface area provided by the soil colloid particles may play a part in perfuser activity.

d). in some cases, judged by the opalescence of the suspension, the number of bacteria did not increase significantly above the original population size. These numbers were probably very low compared with the corresponding soil populations and this alone could account for the low breakdown rates. Failure of the culture population to increase may have been due to inhibition by the herbicide or its breakdown products, or merely to inadequacy of the medium.

No detailed bacteriological study of these mixed cultures was made, but isolations on herbicide/"three salt" agar media tended to produce isolated colonies almost exclusively of the Bacterium globiforme type. On the other hand, inoculation of nutrient agar media (with or without glucose) produced large numbers of colonies of various types.

One attempt to produce 2,4-D breakdown by mixed cultures resulted in apparent failure but produced two other noteworthy results. A soil suspension which had been actively

breaking down 2,4-D, over a long period, was allowed to settle. 50 ml. of the clear supernatant liquid (presumeably containing active bacteria) was pipetted off and made up to 200 ml. with sterile distilled water and 2,4-D to give a final concentration of 100 ppm. This solution was divided equally between four sterile tubes.

Tube a). 50 ml. of suspension only.

Tube b). 50 ml. of suspension + 0.5 ml. of 10% ammonium dihydrogen phosphate (sterile solution).

Tube c). 50 ml. of suspension + 50 gm. of sterile, acid-washed crushed pot.

Tube d). 50 ml. of suspension + 50 gm. of the pot + 0.5 ml. of the phosphate solution.

The solutions were aerated by bubbling with sterile air. 2,4-D assays were carried out at the beginning of the experiment and at intervals up to 50 days. There was no evidence of 2,4-D breakdown in this time. There was, however, a considerable amount of growth in each tube, especially those containing phosphate and particularly in tube d). which also contained crushed pot. Apart from surface effects, the pot may have served as a source of iron and other trace elements. The observed growth would have required an amount of carbon which, if obtained from the 2,4-D, should have produced a detectable change in its concentration. Carbon-dioxide fixation was most unlikely to have accounted for all the growth. A more reasonable explanation is that a partial degradation of the 2,4-D occurred, releasing some carbon for

growth but leaving an intermediate breakdown product which, in some way, maintained the toxicity of the solution towards cress seedlings. Appearance of growth without detectable 2,4-D breakdown had been previously observed in some other mixed suspensions and in some pure culture experiments.

The second interesting observation in this experiment was that the population in the tubes consisted largely of yeast cells (unidentified).

The first observation will be introduced again later when discussing the possible nature of the breakdown products of 2,4-D, etc. (p.198).

Conclusions from pure and mixed culture experiments.

It seems probable that complete degradation of 2,4-D and related compounds requires the co-operation of two or more "types" of micro-organism rather than the lone action of one species. These organisms may, or may not, belong to the same genus, family or wider group. No evidence was produced to show the composition of the active population though a large variety of species was found to persist, even in long active perfusers. It is possible that the active organisms may even have been different strains or mutants of the same species, eg. of the Bacterium globiforme group (which is known to consist of many very closely related forms), for such organisms formed the bulk of isolates on herbicide agar plates.

If it is accepted that 2,4-D breakdown results from a form of symbiotic action, there are two main ways in which this

co-operation could operate:-

a). supply of essential nutrients by species "y" (eg. anti-inhibitors, vitamins, etc.,) to species "x" which enable "x" to continue decomposing 2,4-D. "x" may be unable to synthesise these factors itself from the materials available. If such factors existed they were probably thermolabile.

b). "x", "y", "z", etc., may be members of a chain of species which together can bring about the complete breakdown of 2,4-D, the final catabolic product of "x" being the primary substrate for "y" and so on. Failure of any link in the chain would, at best, result in only partial degradation of the herbicide.

Regardless of whether a). or b). is the operative system, it follows that the only organisms appearing on the 2,4-D isolation plate should be those which can actually use the 2,4-D molecule as a substrate. The other co-operating species in a type a). system would not arise as they would probably require a soil constituent as substrate. The members of a type b). chain could only arise,in order, as the stepwise degradation of 2,4-D proceeded. In either case it is to be expected that breakdown would quickly cease, either for want of essential factors, or because of the accumulation of an intermediate which might act directly, by stimulating the back reaction in a reversible system, or by toxic action on the active organisms.

Poor primary growth and failure of subcultures could be

accounted for in either way. The observation that few colonies, mainly of the one type (B.globiforme), could be isolated on 2,4-D agar from perfusers and the crude, mixed cultures, whereas isolations onto nutrient glucose agar gave rise to very many colonies of various types, is similarly explainable.

If two, or more, species of organisms are concerned in a chain of breakdown reactions for 2,4-D, etc., each one using as substrate the final catabolic product of the preceeding member of the chain, it is a matter for speculation which, if any, of these stages is responsible for the lag phase of the adaptation. The observed lag could be the sum of the partial lags for each stage in the breakdown. It was pointed out earlier (p.54) that B.globiforme type colonies had occasionally arisen as contaminants on uninoculated herbicide agar plates, in less time than the lag period for the particular compound. From this observation two alternatives present themselves, either

a). B.globiforme plays no part in the decomposition process but is merely a common soil organism which,in soil,is resistant to the herbicide. It can maintain a struggling existence on impurities in the agar when other organisms fail; or

b). B.globiforme can accomplish at least the first stage in the degradation of 2,4-D, etc., with apparently little or no adaptation , or lag phase.

Alternative a). is unlikely though it would offer a simple

explanation of the inability to achieve 2,4-D breakdown by pure cultures of Bacterium globiforme isolated in this way. If alternative b). is correct, other assumptions must be made. The first stage in the breakdown is not wholly responsible for the lag and one or other of the decomposition products exerts a delaying effect on the process. Loss of herbicide during the apparent inactivity of the lag phase must be compensated by toxicity or other activity of the intermediates and not detected by the cress test. The apparent inactivity of pure B.globiforme cultures could also be explained on this assumption for, in the absence of other species to continue the decomposition chain, there would be an accumulation of the toxic intermediate(s) which the non-specific cress-test would not be able to distinguish from 2,4-D. Consequently, no detectable breakdown would be recorded. These ideas will be introduced later (p.198,) when discussing the mode of action and possible breakdown products of the herbicides.

Audus (8, 9,) found that a suspension of B.globiforme could break down 2,4-D when perfused over sterile glass-wool or fresh soil. Breakdown over glass-wool suggests that B.globiforme alone could complete the degradation. Lag free breakdown in the presence of fresh soil while not conflicting with the idea of co-operative action, does suggest that the 2,4-D adaptation lag is associated with one of the earlier stages of breakdown accomplished by Bacterium globiforme. The pure cultures used by Audus were much more vigorous than those used in the present research

and it is possible that this vigour may have been the result of a self maintained genetical heterogeneity, the cultures always containing a proportion of morphologically indestinguishable, but metabolically distinct and complementary, mutant forms which were capable of co-operation to bring about the complete degradation of 2,4-D.

Attempts to adapt Pseudomonas fluorescens to 2,4-D breakdown.

Following on the work of Evans (30, 31,), who had chiefly used adapted cultures of Vibrio 01 in studying the breakdown of benzoic acid, phenol and related compounds, Stanier et al (108, 109, 114, 115, 116,) found that cultures of Pseudomonas fluorescens could be readily adapted to break down these and other aromatic substrates. They even produced cell-free enzyme preparations which could bring about some of the breakdown stages in vitro. As Pseudomonas fluorescens was adaptable, with such fascility, to a range of aromatic compounds, it was hoped that it would prove possible to introduce 2,4-D into its repertoire.

Vigorous pure cultures were produced on glucose broth agar and in liquid media. In a series of gradual changes, culture was transferred from glucose broth to glucose/"three salts". Growth on this medium was still good though not quite so vigorous as on the richer medium. In a further series of gradual concentration changes, an attempt was made to lower the glucose concentration to zero and at the same time to increase the 2,4-D concentration of the medium from zero to 0.1%. Subcultures were made at

intervals along the series. Growth continued though the amount decreased as the glucose level fell. In the media containing 2,4-D, but no glucose, growth failed completely.

It would seem that Pseudomonas fluorescens, or at least the particular strain used, cannot be readily adapted to 2,4-D.

Perfusion Results.

Perfusion of various compounds through prepared soil columns constituted the major portion of the practical work in the present research. In general, the same plan of campaign was followed with each compound.

a). Construction of a standard calibration curve. For the phenoxy-acids this was a toxicity/concentration curve, by the cress assay method (p.43) and for the phenols a colour density/concentration curve by the Folin and Ciocalteu method (p.46). For some phenoxy acids only a rough calibration curve was produced but for others, such as 2,4-D and MCPA, the curve was constructed with greater accuracy.

b). Straight perfusion of the simple compound, at low concentrations, through 50 gm. of fresh soil. In this way the duration of the normal lag phase for the compound was determined.

c). Repeated addition of fresh substrate to the enriched perfuser to see if adaptation would be retained over long periods and if the rate of breakdown would change with time.

d). Preparation of other enriched perfusers, from the primarily adapted ones, by taking perfusate from an adapted perfuser and passing it through fresh soil. In this way the transferred enrichment lag was determined and a number of similar, enriched, perfusers quickly produced.

e). Cross perfusion experiments were carried out in which second compounds were perfused through soil already enriched to another compound. If breakdown of the second compound

commenced immediately, or after a lag significantly shorter than the normal one, it was assumed that the soil organisms were simultaneously adapted to the two compounds. Simultaneous adaptation most probably indicates that the two compounds were links in a common reaction chain or that a common structural feature was the point of attack on both molecules. From these experiments it was hoped to obtain clues relating molecular structure to resistance to attack and the point of attack on the molecules.

f). Some experiments were carried out in which mixtures of two, or three, substrates were perfused through either fresh soil, or soil enriched to one or more of the compounds. These experiments were carried out with two objects in mind:-

(i). to see if resistant, or possibly toxic, substances could affect the adaptation to more labile compounds and the rate of breakdown of these same compounds;

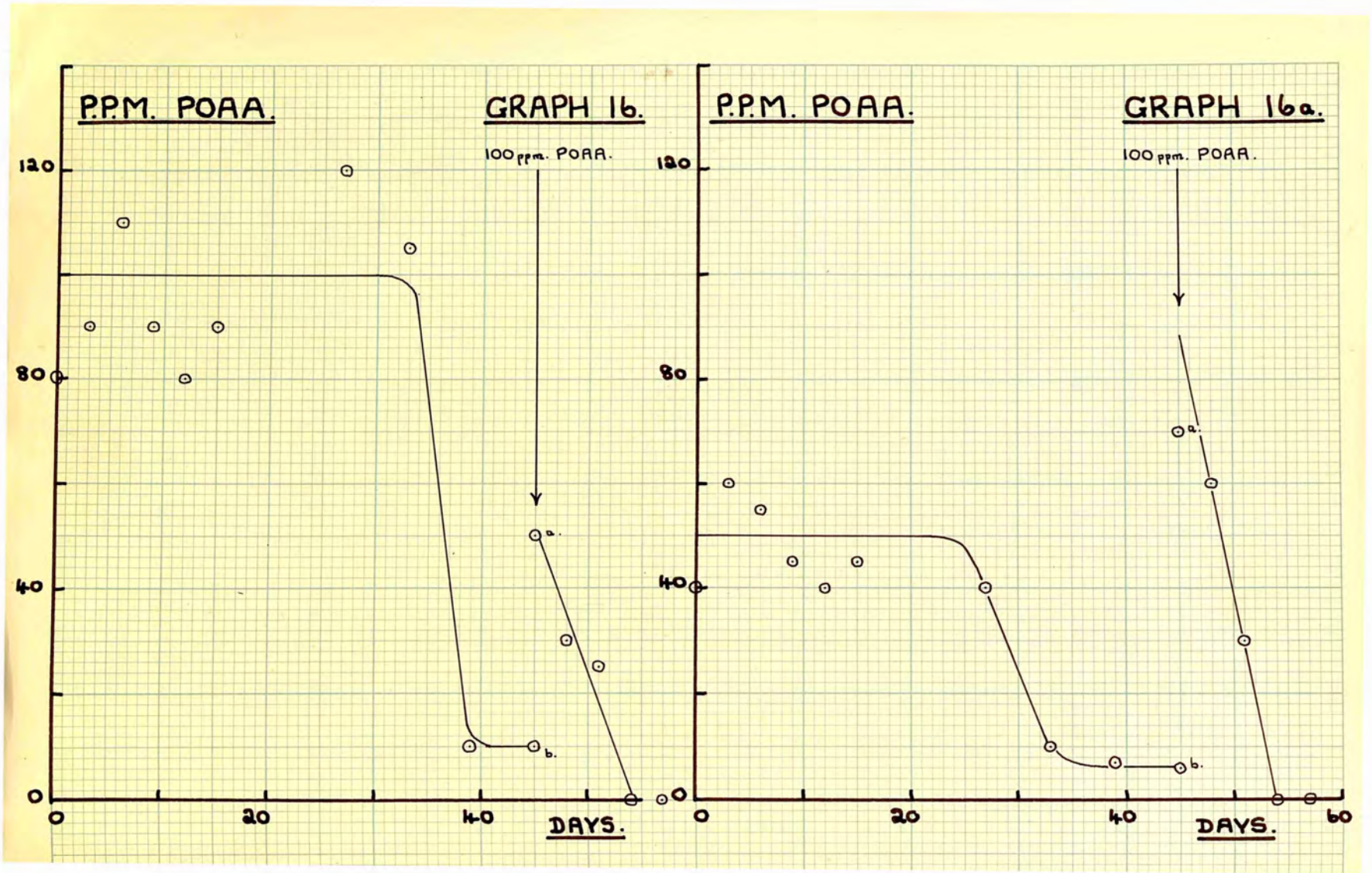
(ii). to see if the lag phase of certain resistant compounds could be shortened by perfusion along with more labile ones.

Some compounds, notably 2,4-D, were studied in more detail and the effects of such factors as concentration, respiratory poisons, phosphate, draining or not draining the perfuser prior to the addition of new substrate, etc., were determined. Retention of adaptation to 2,4-D under various adverse conditions was also observed.

The experimental results are presented in groups, each group being characterised by an initial adaptation compound and the groups arranged in order of

increasing complexity of this compound. Within each group the results are presented in order of increasing complexity of secondary compounds or treatments.

Most of the results are presented in the form of an appendix. Selected derived results and graphical representations are used in the present section.


P.P.M. POAA.
GRAPH 16.
100 ppm. POAA.
120
80
40
0
0
20
40
DAYS.
a.
b.
P.P.M. POAA.
GRAPH 16a.
100 ppm. POAA.
120
80
40
0
0
20
40
60
DAYS.
a.
b.

Phenoxyacetic Acid (POAA).

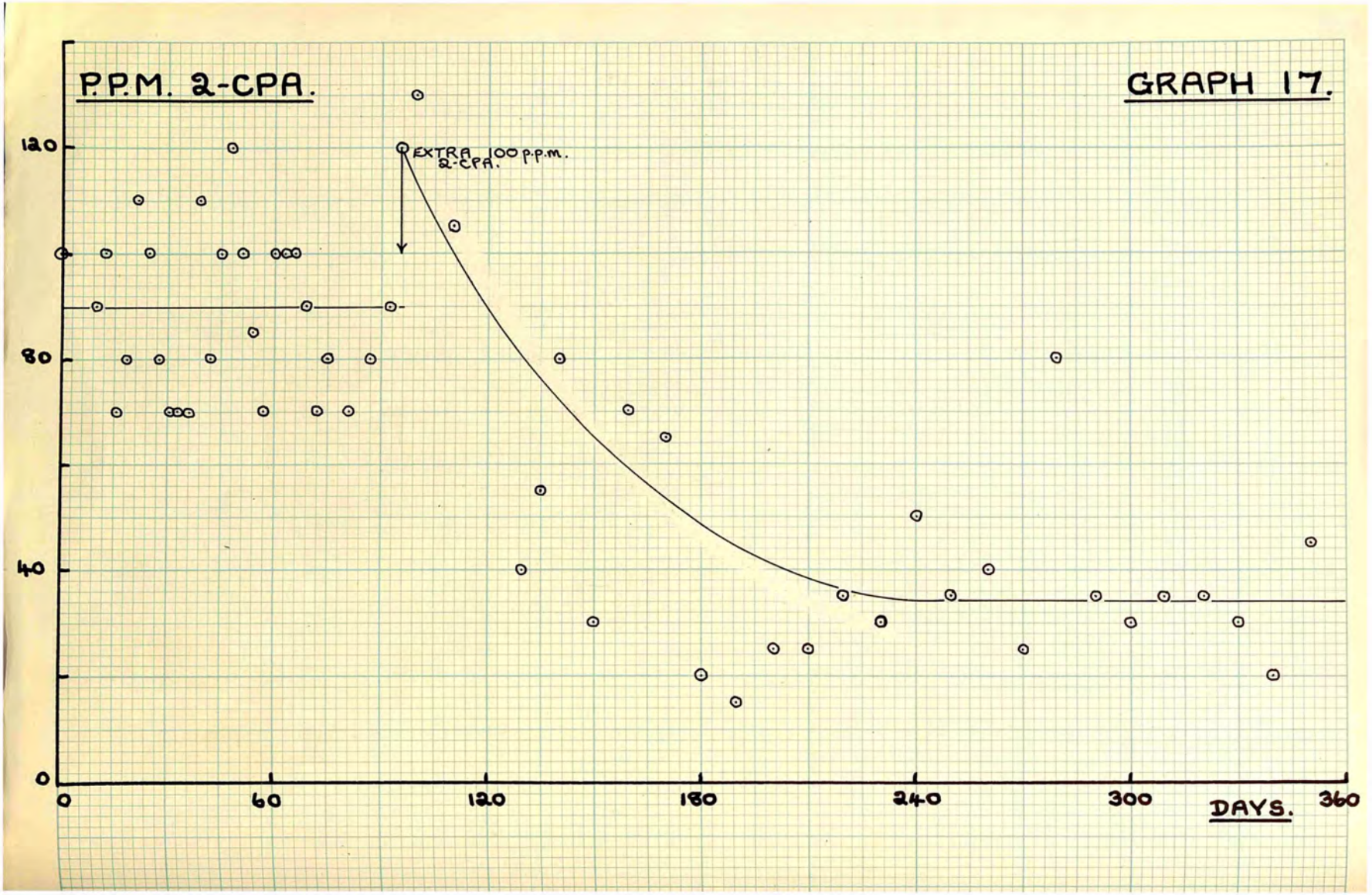
This, the parent compound of the series, has been shown to have a low inhibitory activity by several workers using different tests (84, 85, 85a, 95, 125,

By the Cress Test it was found to have a moderately low activity, about 16.5ppm. being required to produce 50% inhibition. This degree of inhibition was produced by 0.03ppm. of 2,4-D. The Relative Toxicity of POAA was therefore 0.03 x 100 / 16.5 = 0.2 approximately. See also p.44 for explanation of Relative Toxicity.

Direct Perfusion.

The enrichment curves show that adaptation occurred in about 25 days when the perfusate contained 50 ppm. POAA (Graph 16a,) and about 33 days at 100 ppm.(Graph 16,) though these times are probably not significantly different. Breakdown continued with no further lag when the perfusers were drained and refilled with 100 ppm. POAA solution.

There appears to be no simple explanation of the slight residual toxicity at the close of the primary perfusions, for there were no residues from the refills.


P.P.M. 2-CPA.
GRAPH 17.
EXTRA 100 p.p.m. 2-CPA.
120
80
40
0
0
60
120
180
240
300
360
DAYS.

2-chlorophenoxyacetic acid (2-CPA).

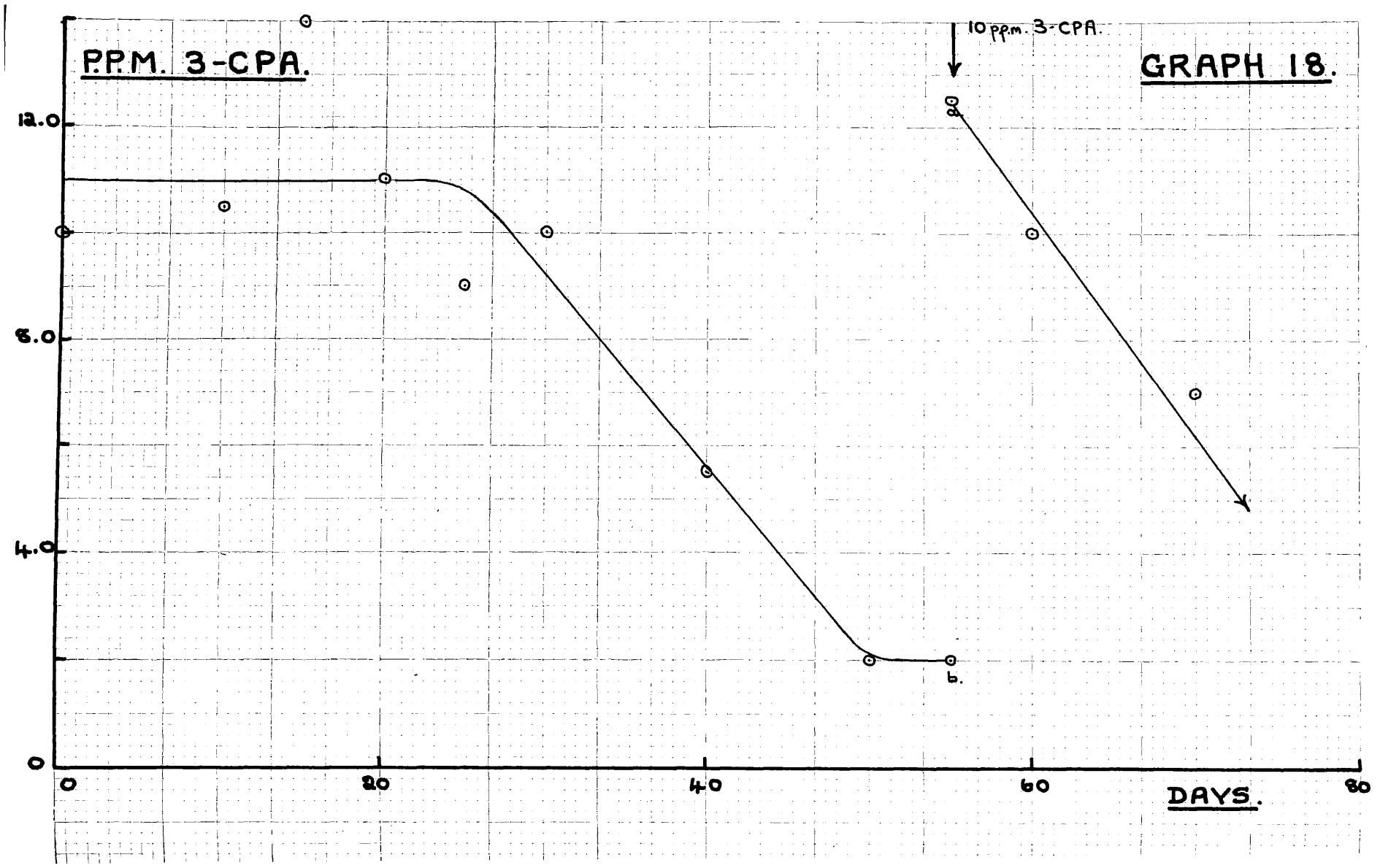
Though still of low physiological activity, 2-CPA has been found to be more active than phenoxyacetic acid (84, 85, 85a, 95, 125,).

The Cress Test showed it to be more toxic than POAA with a Relative Toxicity of approximately 4.

Direct Perfusion.

Three separate perfusers were run with 2-CPA, each time at 100 ppm. and using fresh soil. They were carried on for 205, 350 and 350 days respectively. (Graph 17, Tables 17, 17a and 17b). After long periods of running, it was found neccessary to make good sampling and other losses by topping up the perfusate with 100 ppm. 2-CPA solution.

Because of the somewhat erratic behaviour of the assays, it was impossible to determine accurately the fate of the 2-CPA. Definite adaptation, such as occurred in 2,4-D perfusions, did not seem to take place with 2-CPA even though perfusion was very prolonged. There appeared to be a gradual disappearance of 2-CPA from the perfusate, either by breakdown or some other process, but at no time did the apparent concentration fall below 25% of the initial value. It is possible that some microbial, or purely chemical, breakdown had occurred giving a product toxic to the cress and also inhibiting further breakdown by the soil organisms. The apparent lack of adaptation, slow breakdown rate and erratic assay behaviour of the perfusate samples may be accounted for in this way.


P.P.M. 3-CPA.
GRAPH 18.
10 p.p.m. 3-CPA.
12.0
8.0
4.0
0
0
20
40
60
80
DAYS.
5.

3-chlorophenoxyacetic acid (3-CPA).

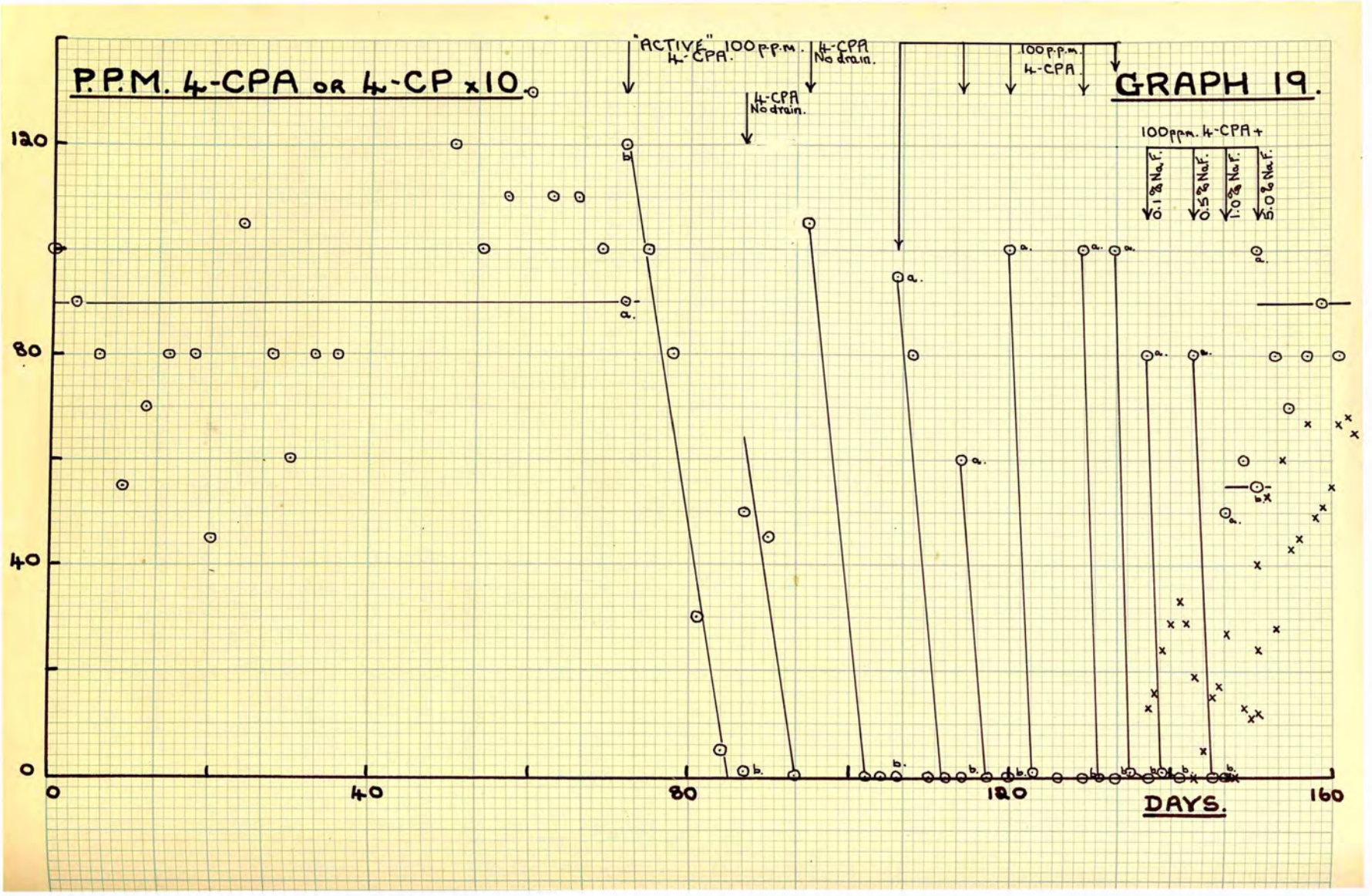
On the whole, this compound has been found to have a fairly high physiological activity (63, 84, 85a, 95,) though the level of activity seems to depend on the test method employed (125,).

Relative Toxicity by the Cress Test was found to be approximately 4, the same value as for 2-CPA, and still a low figure.

Direct Perfusion.

At 100 ppm. (Table 18a) there was no evidence of significant breakdown in 70 days perfusion. With 10 ppm. (Graph and Table 18) a typical enrichment occurred after only 30 days perfusion though, even after 50 days, there appeared to be a residual activity equivalent to about 20% of the initial 3-CPA content. After draining and refilling the perfuser with 10 ppm. 3-CPA, breakdown commenced immediately but at the same slow rate.

The typical enrichment curve obtained with 10 ppm. 3-CPA proved that adaptation to the compound by soil micro-organisms could occur. The apparent lack of adaptation at 100 ppm. must have been due to inhibition of these organisms. 3-CPA itself may be toxic at higher concentrations or intermediate breakdown products, which might be expected to accumulate in larger amounts from 100 ppm. 3-CPA, may be responsible. In either case breakdown might be expected to cease before the herbicide level has been lowered sufficiently to give a significantly different assay result.


P.P.M. 4-CPA or 4-CP x10.
GRAPH 19.
"ACTIVE" 4-CPA.
100 p.p.m. 4-CPA
4-CPA No drain.
4-CPA No drain.
100 p.p.m. 4-CPA
100 ppm. 4-CPA +
0.1% Naf.
0.5% Naf.
1.0% Naf.
5.0% Naf.
120
80
40
0
0
40
80
120
160
DAYS.

4-chlorophenoxyacetic acid (4-CPA).

4-CPA has been stated to have a medium (63,) or high (85a, 95, 125,) physiological activity.

The Cress Test showed it to have the very high Relative Toxicity of 100, ie. as toxic to cress as 2,4-D.

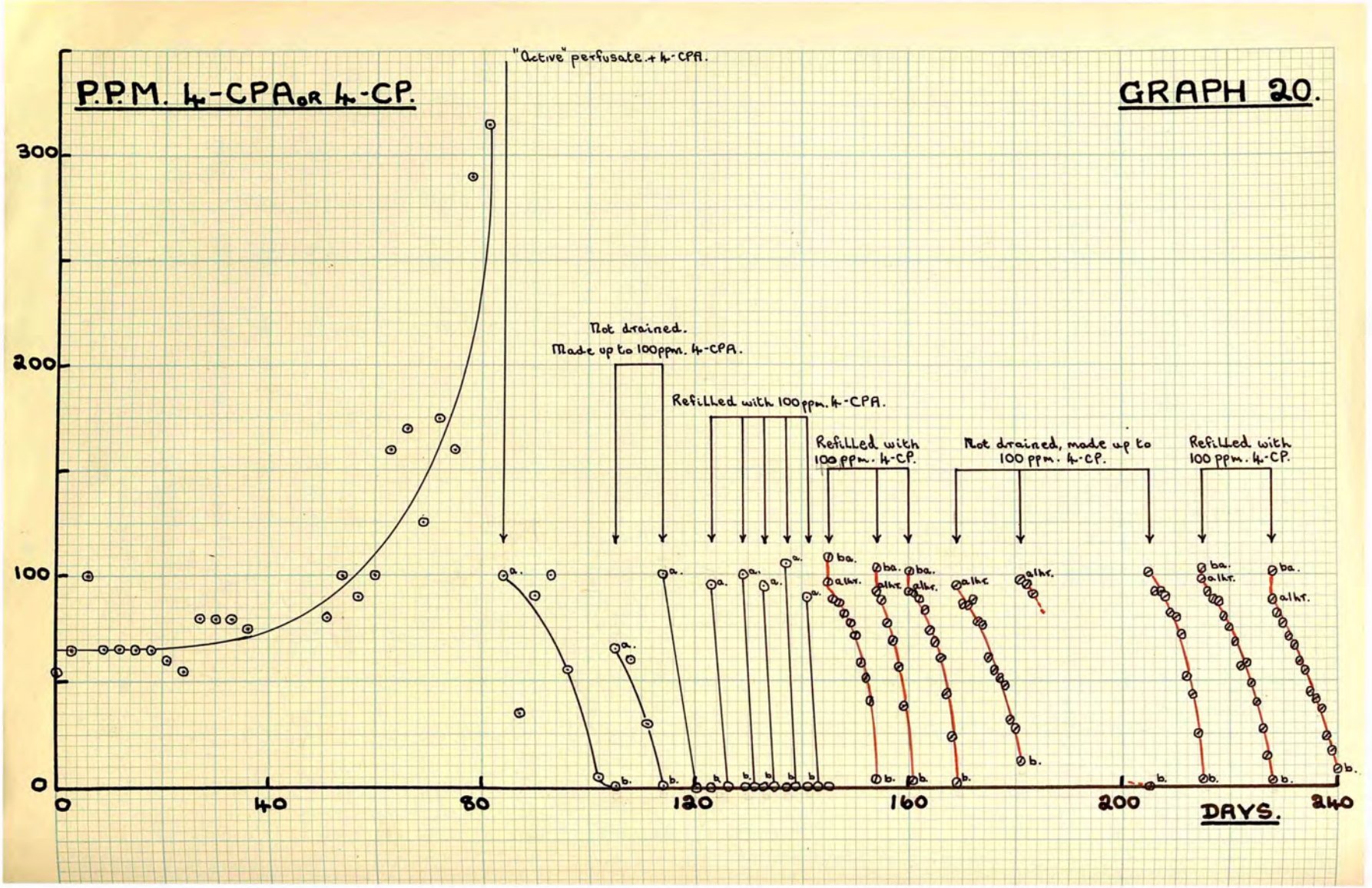
Direct Perfusion.

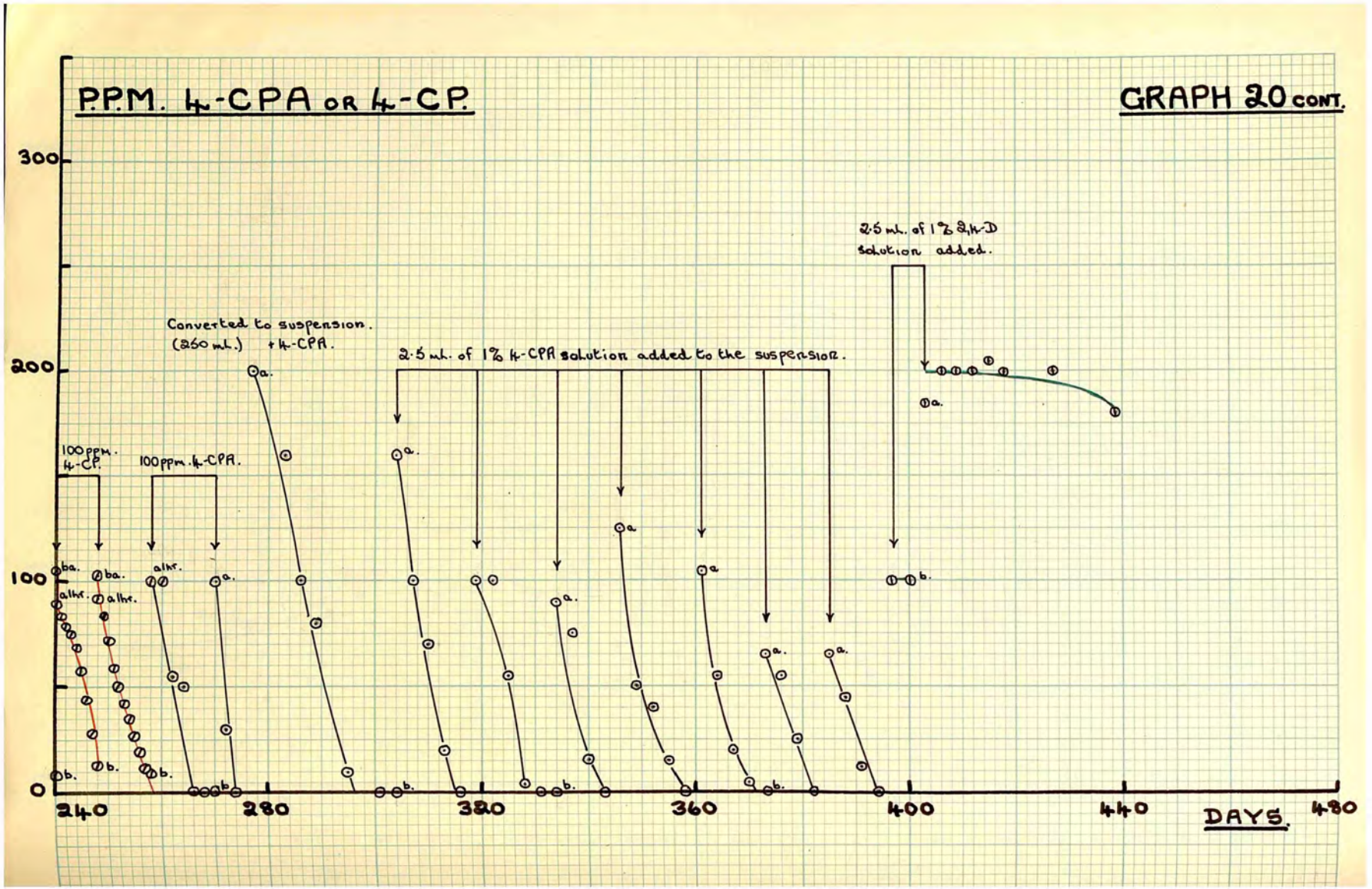
4-CPA proved to be a very labile compound in soil. Perfusions were only carried out at 100 ppm. and the lag phase proved to be short. Values of 6 to 12 days were obtained for the lag with 8 or 9 days as the commonest values (Graphs 21, 23, 24, 25, 26, 27; Tables 21, 23, 23a, 23b, 23c, 24, 25, 26, 27, 27a, 27b, 27c,). High rates of breakdown were maintained by repeated drainage of the perfusers and refilling with fresh substrate. Because of the short lag phase, and the ease with which enriched perfusers could be produced, it was never necessary to resort to the transferred adaptation technique when dealing with soil.

Transfered Adaptation to Broken Pot.

Two attempts were made to produce enriched populations in perfusers containing sterile, broken flower-pot in place of the usual soil. Fresh soil was shaken with sterile water, allowed to settle and the clear extract decanted off. From this, 500 ml. of 100 ppm. 4-CPA was prepared and divided equally between the two perfusers (Graphs and Tables 19 and 20).

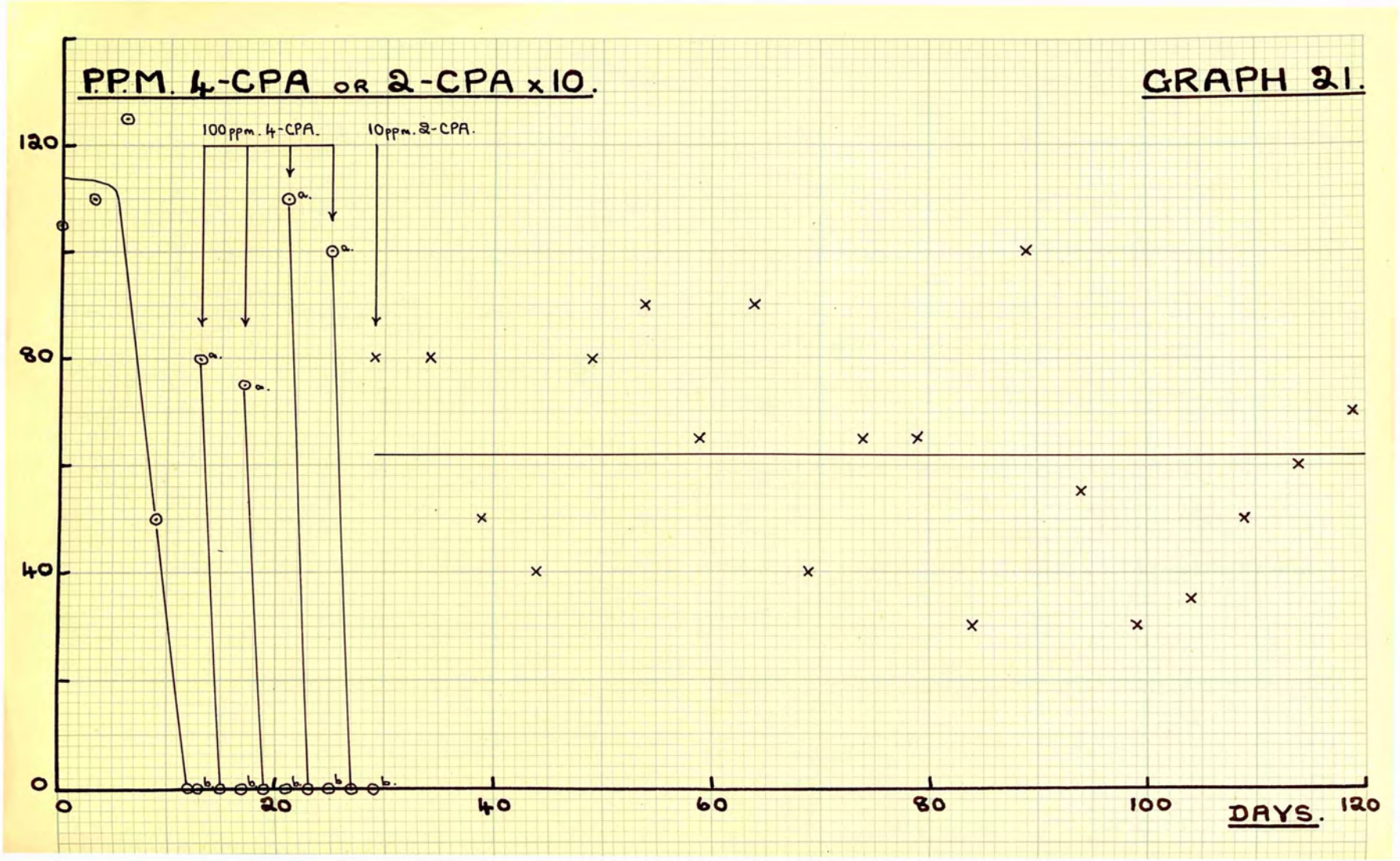
In the first perfuser (Graph and Table 19) there was no evidence of adaptation in 72 days. At this point the perfuser was drained and refilled with a 100 ppm. solution of 4-CPA prepared from the spent perfusate from a 4-CPA enriched soil perfuser. Breakdown commenced almost immediately and was completed in about 10 days. Activity was maintained, and the rate of breakdown gradually increased, by repeatedly adding new 4-CPA to the perfuser, with or without drainage and change of perfusate. Such behaviour suggests that a small number of adapted bacteria had been transferred to the perfuser and that this active population gradually increased in size, most probably on the large surface area of the crushed pot. When the perfuser was finally dismantled, the pot was found to be thickly coated with a slimy material. During the later stages of this perfusion, the effect of fluoride poisoning on 4-CPA breakdown was investigated. 0.1% and 0.5% NaF were apparently ineffective but 1% probably, and 5% NaF certainly, did inhibit breakdown of the herbicide. As these concentrations are much higher than those normally required to inhibit respiratory and other processes, it seems unlikely that 4-CPA is decomposed oxidatively in the soil and that the fluoride inhibition resulted from a general poisoning of the bacterial protoplasm. Though there was some evidence of accumulation of substances giving a phenol positive reaction, during the period of fluoride poisoning, the amounts were not significant and may have been artefacts caused by a). interference in the test by the high fluoride

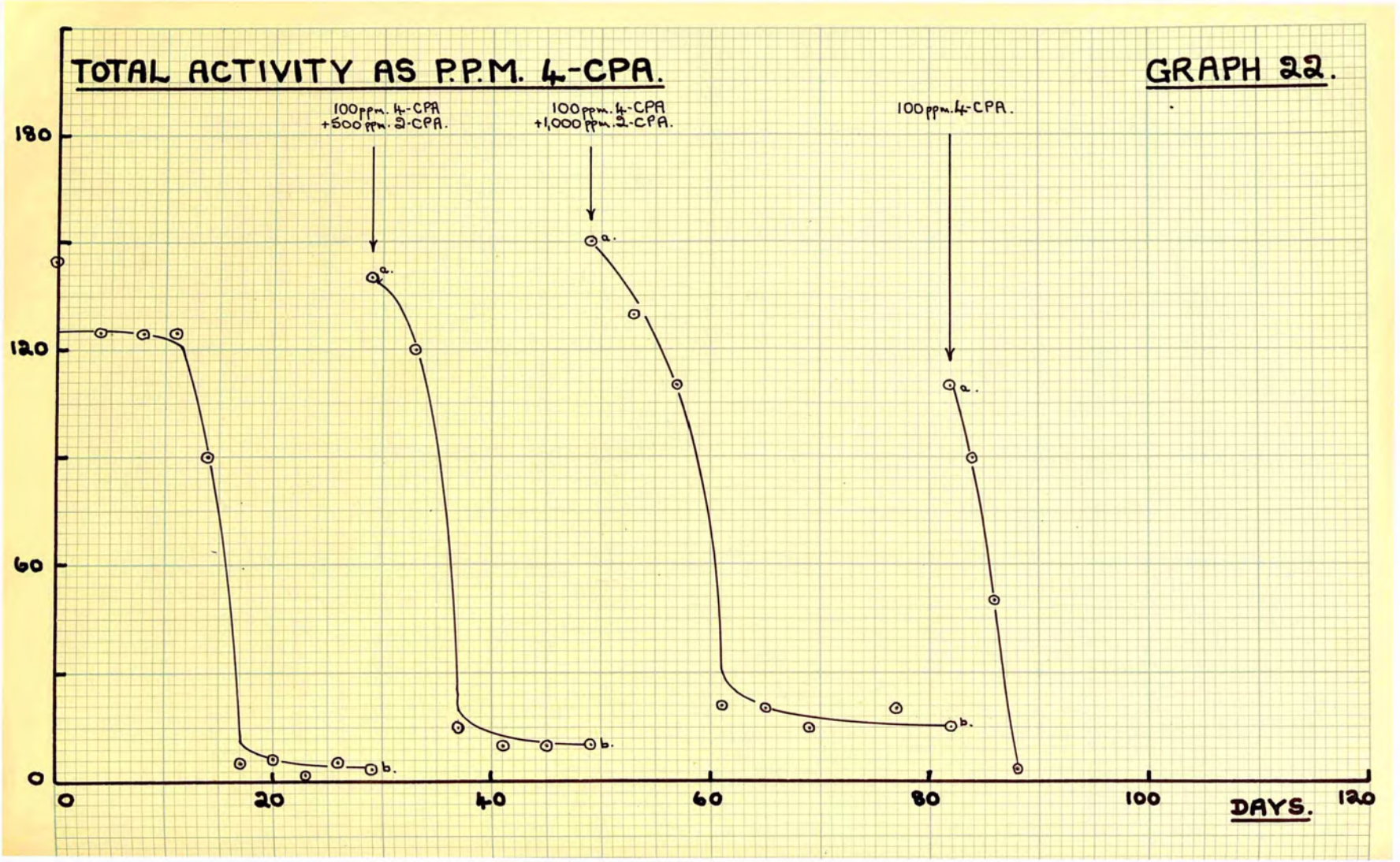

P.P.M. 4-CPA OR 4-CP.
GRAPH 20.
"Active" perfusate + 4-CPA.
Not drained.
Made up to 100ppm. 4-CPA.
Refilled with 100ppm. 4-CPA.
Refilled with 100 ppm. 4-CP.
Not drained, made up to 100 ppm. 4-CP.
Refilled with 100 ppm. 4-CP.
300
200
100
0
0
40
80
120
160
200
240
DAYS.


P.P.M. 4-CPA OR 4-CP.
GRAPH 20 CONT.
300
200
100
0
100 ppm. 4-CP.
100 ppm. 4-CPA.
Converted to suspension. (250 ml.) + 4-CPA.
2·5 ml. of 1% 4-CPA solution added to the suspension.
2·5 ml. of 1% 2,4-D solution added.
a.
b.
a hr.
240
280
320
360
400
440
480
DAYS.

concentration, or b). phenol positive substances produced by the action of fluoride on the organic debris of the perfuser. Sodium fluoride was not inhibitory to cress seeds till its concentration in the assay solution reached 0.05%. Consequently it only interfered in the 1 ppm. dilution.

With the second crushed pot perfuser (Graph and Table 20) the time / concentration curve did not follow the normal inactivity or adaptation courses but, instead, showed an apparent increase in herbicide concentration. Part of the rise may be attributable to concentration of the perfusate by evaporation but this was not sufficient to account for the whole increase. It is unlikely that the bacterial population of the soil extract was qualitatively different from that of the soil though it may have become modified by the extinction of some species unable to resist the herbicide in the absence of soil. If one or more of these species was concerned with later stages of 4-CPA breakdown, it is possible that a normal first stage adaptation and slow enrichment occurred but that incomplete degradation resulted. If the accumulating breakdown products were herbicidaly more active than 4-CPA, the perfusate concentration as measured by assay would appear to rise. When the perfuser was drained and refilled, with 100 ppm. 4-CPA in active spent perfusate, bacteria must have been added which could bring about complete breakdown for, from that point, the perfuser behaved in the normal way with the rate of breakdown increasing with successive refills. At a much later stage of this perfusion


P.P.M. 4-CPA OR 2-CPA x 10.
GRAPH 21.
100 ppm. 4-CPA.
10 ppm. 2-CPA.
120
80
40
0
0
20
40
60
80
100
120
DAYS.


TOTAL ACTIVITY AS P.P.M. 4-CPA.
GRAPH 22.
100ppm. 4-CPA +500ppm. 2-CPA.
100ppm. 4-CPA +1,000ppm. 2-CPA.
100ppm. 4-CPA.
180
120
60
0
a.
b.
0
20
40
60
80
100
120
DAYS.

the accumulation of organic slime on the broken pot became so great that it ceased to function efficiently. Perfusate and pot were shaken together and the liquid drained into a sterile flask. This bacterial suspension continued to break down 4-CPA, though at a somewhat reduced rate, when incubated at 28°C.

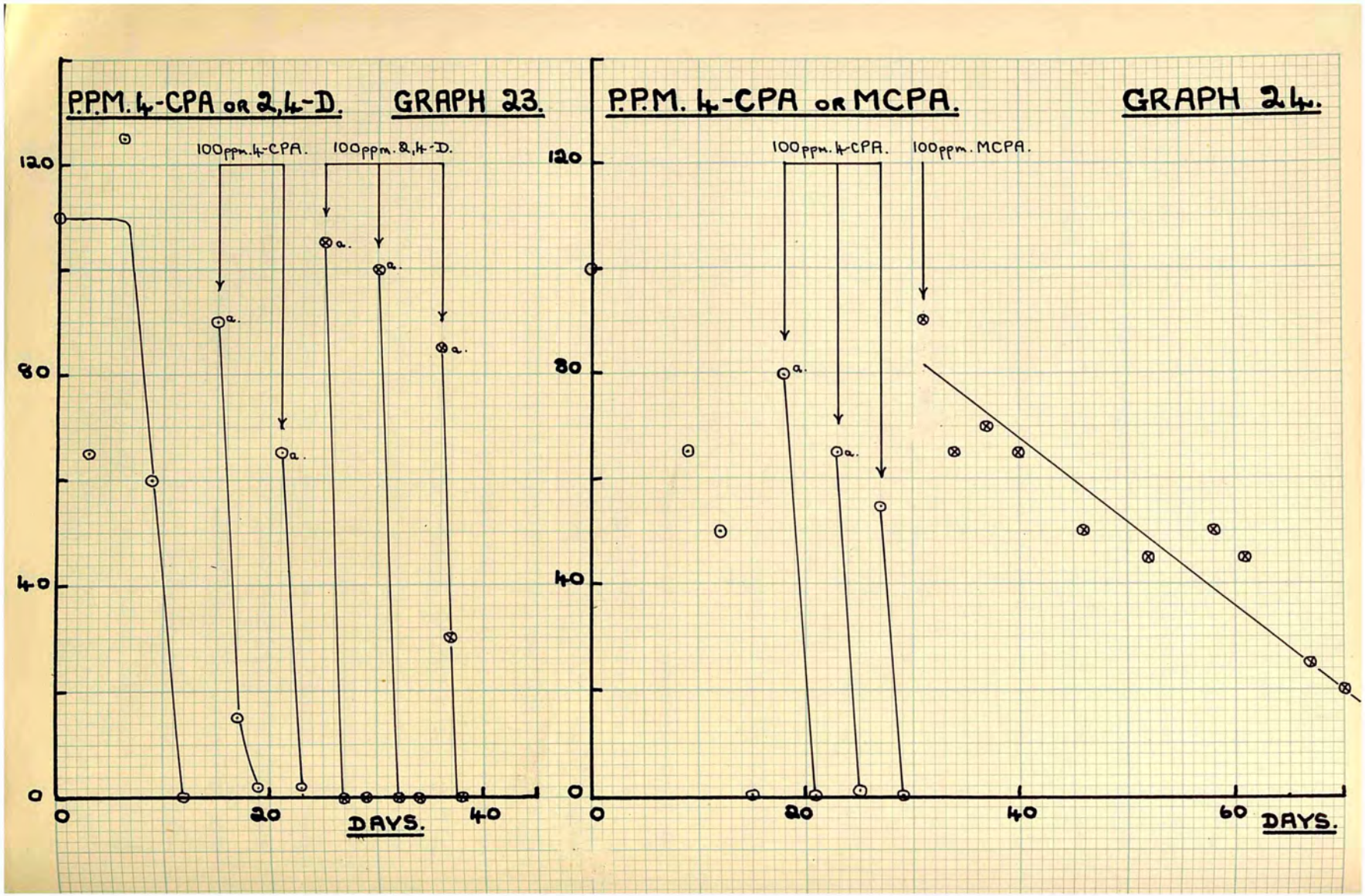
4-CPA followed by 2-chlorophenoxyacetic acid (2-CPA).

2-CPA was perfused at 10 ppm. through a soil column enriched to 100 ppm. 4-CPA. The assay results were very erratic but suggested that no breakdown occurred in 90 days (Graph and Table 21).

4-CPA/2-CPA mixtures.

A mixture containing 100 ppm. of each of these compounds proved more toxic in the assay test than could be expected by simple summation of the individual toxicities. This also occurred when higher ratios of 2-CPA to 4-CPA were tested and suggested synergistic action.

On perfusion, the 100 ppm./100 ppm. mixture invoked an adaptation to the 4-CPA component, after a lag slightly longer than normal (Graph 22, Tables 22, 22a,). With subsequent refills 500 and 1,000 ppm. 2-CPA failed to prevent breakdown of 100 ppm. 4-CPA though the breakdown rate was slower, especially at the 1,000 : 100 level. The inhibition was probably of the specific enzyme system concerned with 4-CPA breakdown, rather than a general toxicity towards the entire adapted population, for, on draining and refilling with 100 ppm. 4-CPA only, a higher rate of breakdown was achieved.

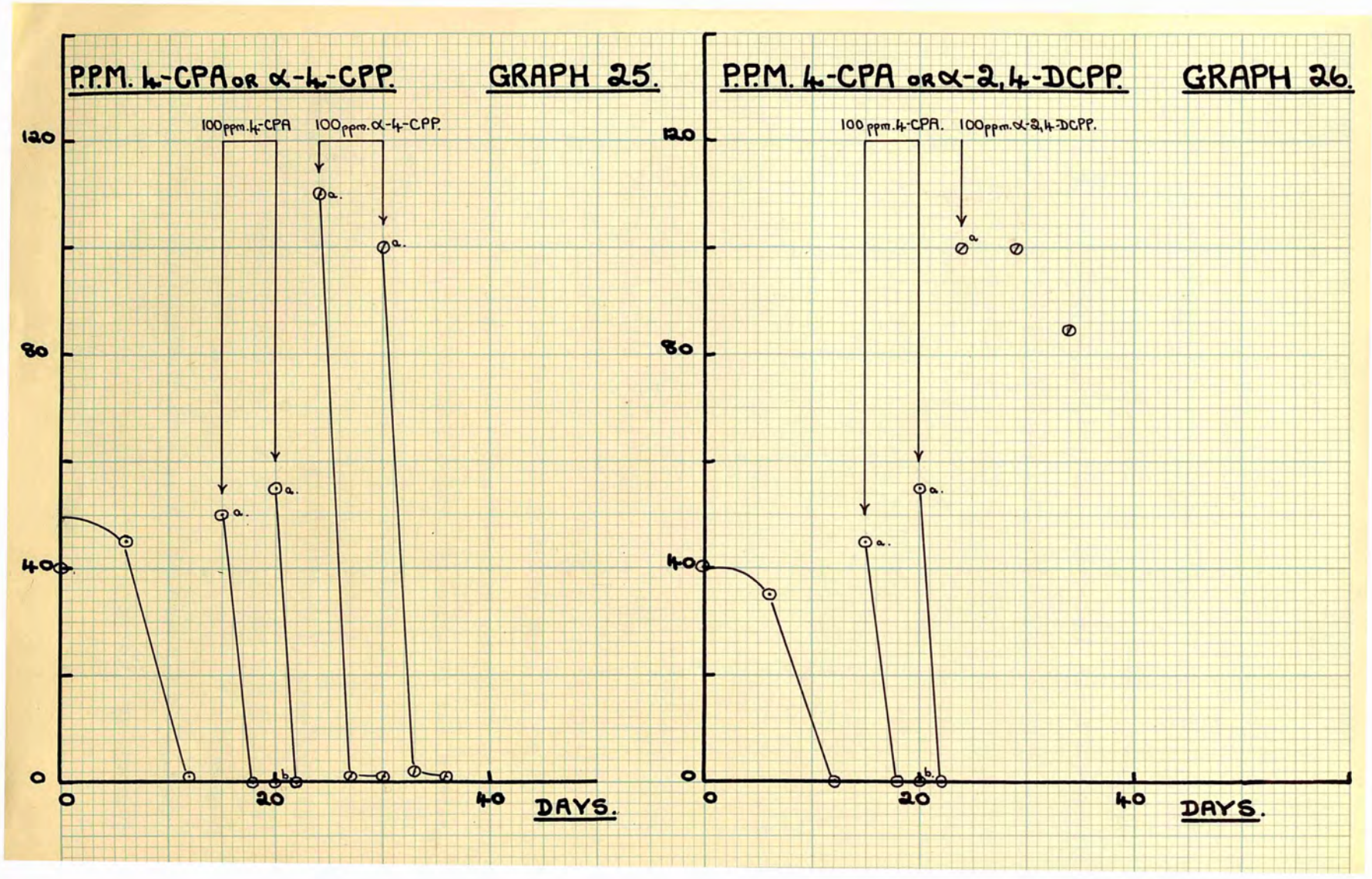

P.P.M. 4-CPA OR 2,4-D.
GRAPH 23.
100ppm. 4-CPA.
100ppm. 2,4-D.
120
80
40
0
0
20
40
DAYS.
P.P.M. 4-CPA OR MCPA.
GRAPH 24.
100ppm. 4-CPA.
100ppm. MCPA.
120
80
40
0
0
20
40
60
DAYS.

The rate was still lower than normal. If it is assumed that 50 ml. of perfusate was retained by the soil column when it was drained, then the 2-CPA remaining in this perfusate would dilute to 1/6 of its concentration,ie. approximately 150 ppm.,when the 250 ml. of 4-CPA was added. This concentration of 2-CPA would still be high enough to reduce the 4-CPA breakdown rate below its uninhibited level. The inhibiting action of 2-CPA may have due to its absorbtion, competitively, onto the sites of 4-CPA breakdown. There was no evidence of bacterial action on the 2-CPA itself, for the mixed perfusate activity tended to fall each time to a level equivalent to that expected from the 2-CPA component alone, or to a slightly higher level suggesting some residual 4-CPA.

4-CPA followed by 2,4-dichlorophenoxyacetic acid (2,4- D).

In each case (Graph 23, Tables 23, 23a, 23b, 23c,) breakdown of 2,4-D commenced immediately, and at a rapid rate, when added at 100 ppm. to a perfuser enriched towards 100 ppm. of 4-CPA. The high rate of breakdown was maintained through several changes of perfusate.

In contrast, when 2,4-D was added to a crude bacterial suspension (prepared ffom the growth in a crushed pot perfuser, Day 277, Table 20,) active against 100 ppm. 4-CPA, it was not affected at the 100 ppm. level in 6 days. The concentration was then increased to 200 ppm. 2,4-D and remained unaltered at this level for a further 36 days. (Graph and Table 20.).


P.P.M. 4-CPA OR α-4-CPP.
GRAPH 25.
100 ppm. 4-CPA
100 ppm. α-4-CPP.
120
80
40
0
a.
b.
0
20
40
DAYS.
P.P.M. 4-CPA OR α-2,4-DCPP.
GRAPH 26.
100 ppm. 4-CPA.
100 ppm. α-2,4-DCPP.
120
80
40
0
a.
b.
0
20
40
DAYS.

A further suspension (prepared from the above) active against 100 ppm. 4-CPA also showed, in 6 days, an amount of 2,4-D breakdown of doubtful significance (Table 20a).

4-CPA followed by 4-chloro,2-methylphenoxyacetic acid (MCPA).

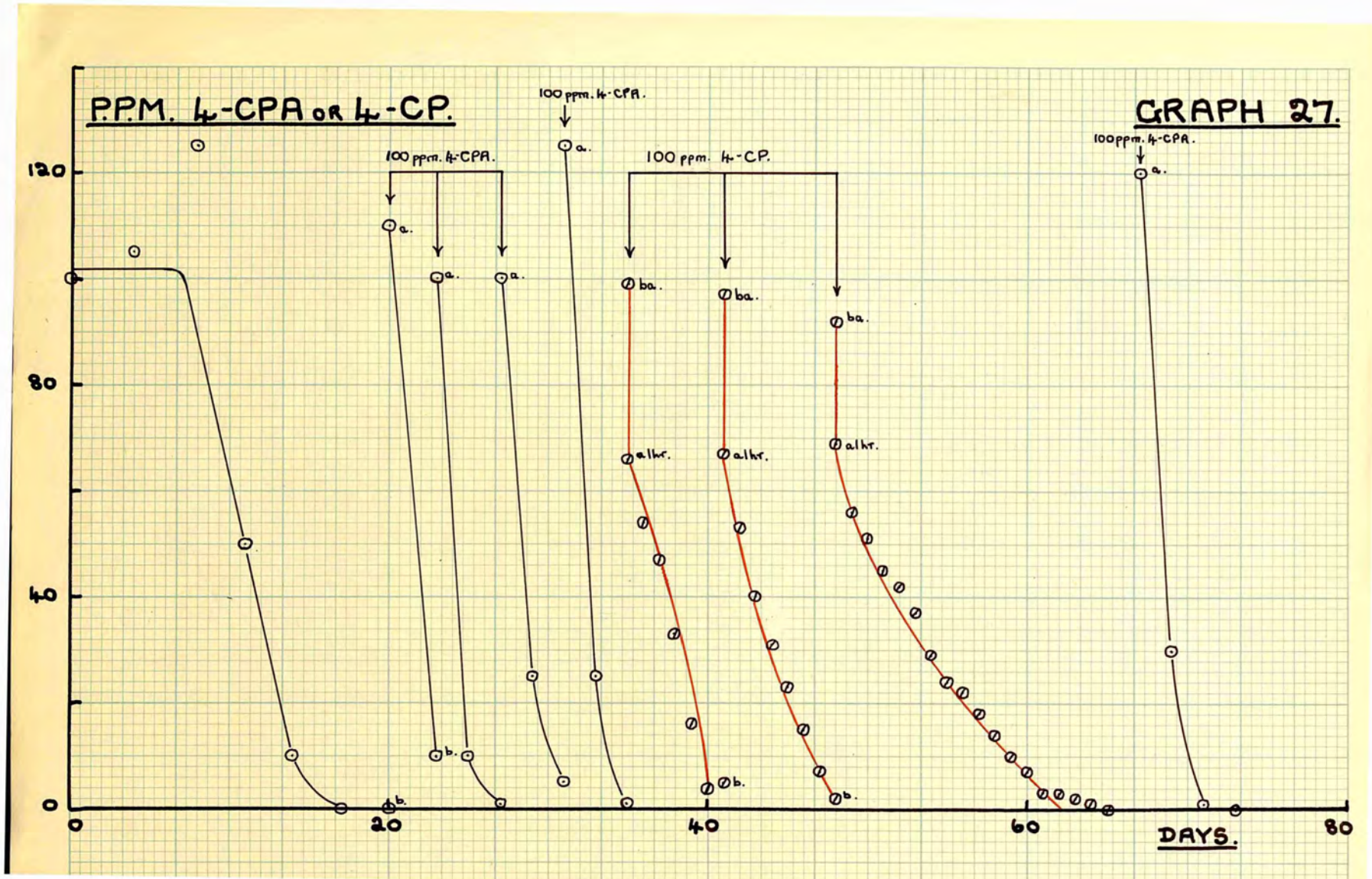
Although 100 ppm. MCPA was broken down when added to an active 4-CPA (100 ppm.) perfuser, the rate was very slow and the perfuser was not refilled (Graph and Table 24).

4-CPA followed by ∝-4-chlorophenoxypropionic acid (∝-4-CPP).

Breakdown commenced immediately and continued at a rapid rate when 100 ppm. ∝-4-CPP was added to an active 4-CPA (100 ppm.) perfuser. The process was repeated after draining and refilling (Graph and Table 25). In both instances the perfusate retained a slight residual activity after the initial rapid breakdown phase had ended. This is consistent with the hypothesis, developed later, that only one of the enantiomorphs is broken down leaving the second, less physiologically active, isomer unaffected in the perfusate.

4-CPA followed by ∝-2,4-dichlorophenoxypropionic acid.

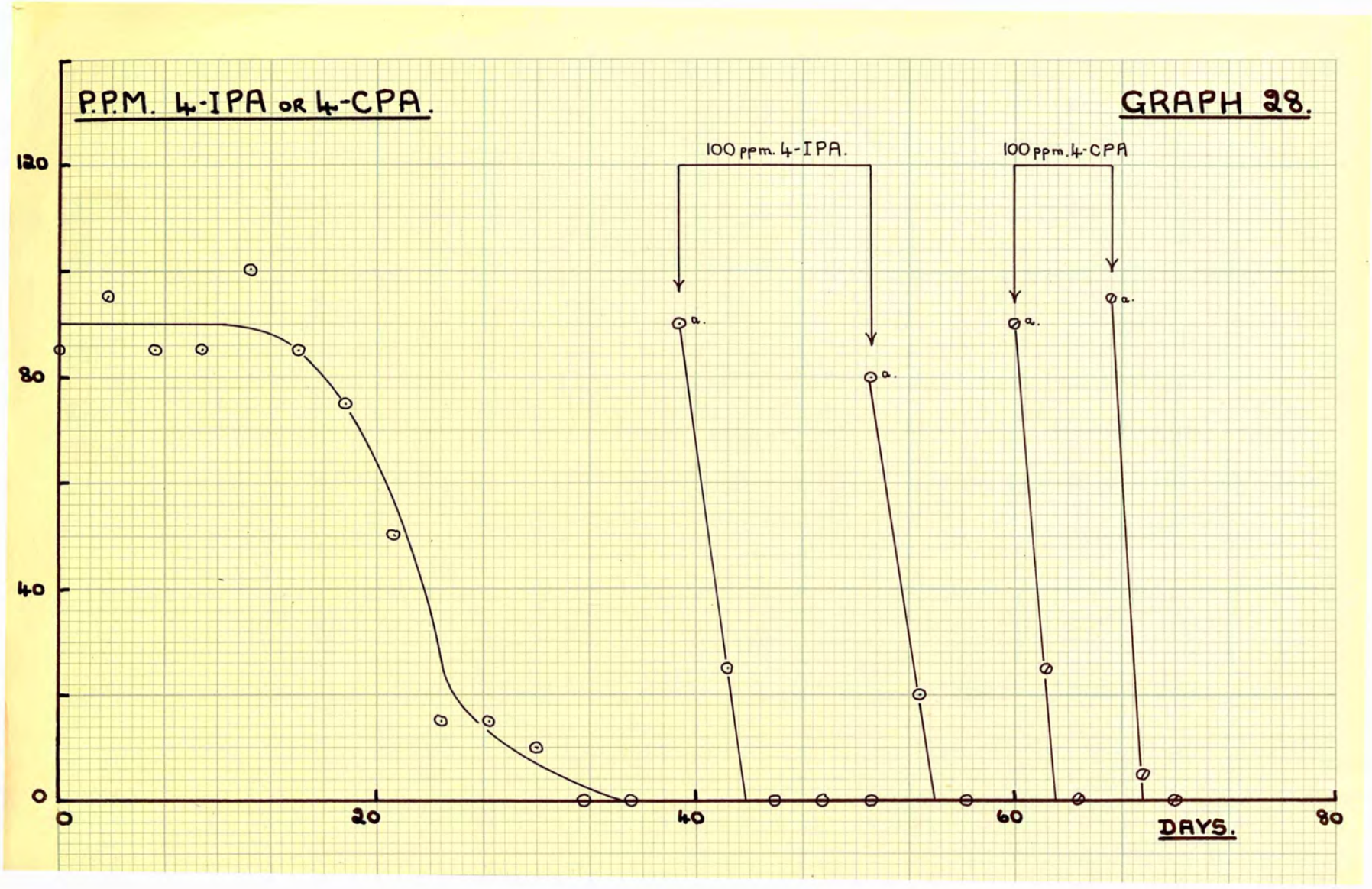
There was some evidence of breakdown of ∝-2,4-DCPP (100 ppm.) in 10 days after adding it to a 4-CPA (100 ppm.) enriched perfuser. It was not possible to continue the perfusion, nor to attempt a repeat, so the result must be regarded as inconclusive (Graph and Table 26).


P.P.M. 4-CPA OR 4-CP.
GRAPH 27.
100 ppm. 4-CPA.
100 ppm. 4-CPA.
100 ppm. 4-CP.
100ppm. 4-CPA.
a.
ba.
a1hr.
b.
120
80
40
0
0
20
40
60
80
DAYS.

4-CPA followed by 4-chlorphenol (4-CP).

In fresh soil, 4-CP concentration tended to fall to about 20% of its initial level but adaptation was slow (see Graph 68, Tables 68, 68a,).

Each time 4-CP, at 100 ppm., was added to a 4-CPA enriched perfuser it was readily broken down (Graph 27, Tables 27, 27a, 27b, 27c,). With two of the perfusers (Graph 27, Tables 27, 27a,) there was some tendency to loss of activity but in the other two (Tables 27b, 27c,) the rapid rate of breakdown was maintained over many renewals of perfusate. (cf. 2,4-D/2,4-DCP perfusers, Graphs 41,41a, 42, Tables 30d,30e,30f, 38a, 41,41a,41b,41c,41d,41e, 42,42f,). The 4-CPA enriched perfuser based on flower-pot (Graph 20) was also found to be active against 4-CP (100 ppm.) though the breakdown rate was slower than that over soil. Retention of adaptation to 4-CPA during active 4-CP breakdown was shown by draining three perfusers (Graphs 20 and 27, Tables 20, 27 27a,) and refilling with the former compound. In each one the 4-CPA disappeared quickly and without lag.


P.P.M. 4-IPA OR 4-CPA.
GRAPH 28.
100 ppm. 4-IPA.
100 ppm. 4-CPA
a.
120
80
40
0
0
20
40
60
80
DAYS.

4-iodophenoxyacetic acid (4-IPA).

Muir and Hansch (85a,) found this compound to be inactive in promoting cell elongation. The Cress Test showed it to have a low herbicidal activity, the Relative Toxicity being approximately 5.

Direct Perfusion.

At 100 ppm. this followed a fairly normal course, adaptation occurring in about 15 days (Graph and Table 28,). This was about 1.5 to 2 times as long as the 4-CPA lag. A high breakdown rate was maintained through three consecutive drainings and refillings at 100 ppm. of 4-IPA.

4-IPA followed by 4-chlorophenoxyacetic acid (4-CPA).

The 4-IPA perfuser (Graph and Table 28) was drained and refilled with 100 ppm. 4-CPA. A rapid rate of breakdown was observed with no obvious lag.

2,4-dichlorophenoxyacetic acid (2,4-D).

Many workers using a variety of test methods have shown 2,4-D to have a high physiological activity (for extensive literature references see 10, 125, 127,). Others have studied its disappearance from soil under various natural and artificial conditions and using various methods to indicate its presence or absence (2, 3, 4, 7, 8, 9, 11, 16, 17, 23, 26, 27, 28, 42, 43, 44, 47, 48, 49, 56, 57, 57a, 59, 62, 77, 81, 86, 88, 89, 90, 91, 92, 97, 123, 126, 131,). Though all found it to be fairly labile in soil, widely scattered values were obtained for the persistence or lag phase. Audus (9,) using the Perfusion Technique and Cress Assay showed that the lag was about 14 days for 10 and 100 ppm. solutions. Throughout the present work, 2,4-D has been taken as the standard of toxicity and allocated the Relative Toxicity value of 100.

Adaptation and General Perfusion Phenomena.

Adaptation and enrichment to 2,4-D proved to be a simple and reliable process and the established adapted populations proved very stable.

When the perfusate contained 100 ppm. 2,4-D, adaptation normally took place in about 10 to 14 days though some took slightly longer (Graphs 32, 33, 36, and Tables 32, 33, 33a, 36, 42b, 42f,). A larger perfuser containing 4 litres of perfusate and 750 gms. of soil in place of the usual 50 gms. (Table 31.) had a lag of similar duration (about 16 days).

By the transferred adaptation process the initial breakdown lag was considerably reduced, being always less than 10 days and often less than 6 days (Graphs 35, 37, 42a, 42c, Tables 30f, 33b, 33c, 35, 35a, 35b, 35c, 37, 37a, 42a, 42c, 42d, 42e,).

With increased perfusate strengths, up to 1,000 ppm., an increase in duration of the lag was observed (Graphs 30, 41, 41a, 42, and Tables 30, 30d, 30e, 30f, 38a, 41, 41a, 41d, 42,). Adaptation usually occurred in the 16 to 20 day range though the exact time was often not determined. Transferred enrichment at high concentrations followed the same general pattern, of reduced lag, as at lower concentrations (Tables 41b, 41c, 41e,).

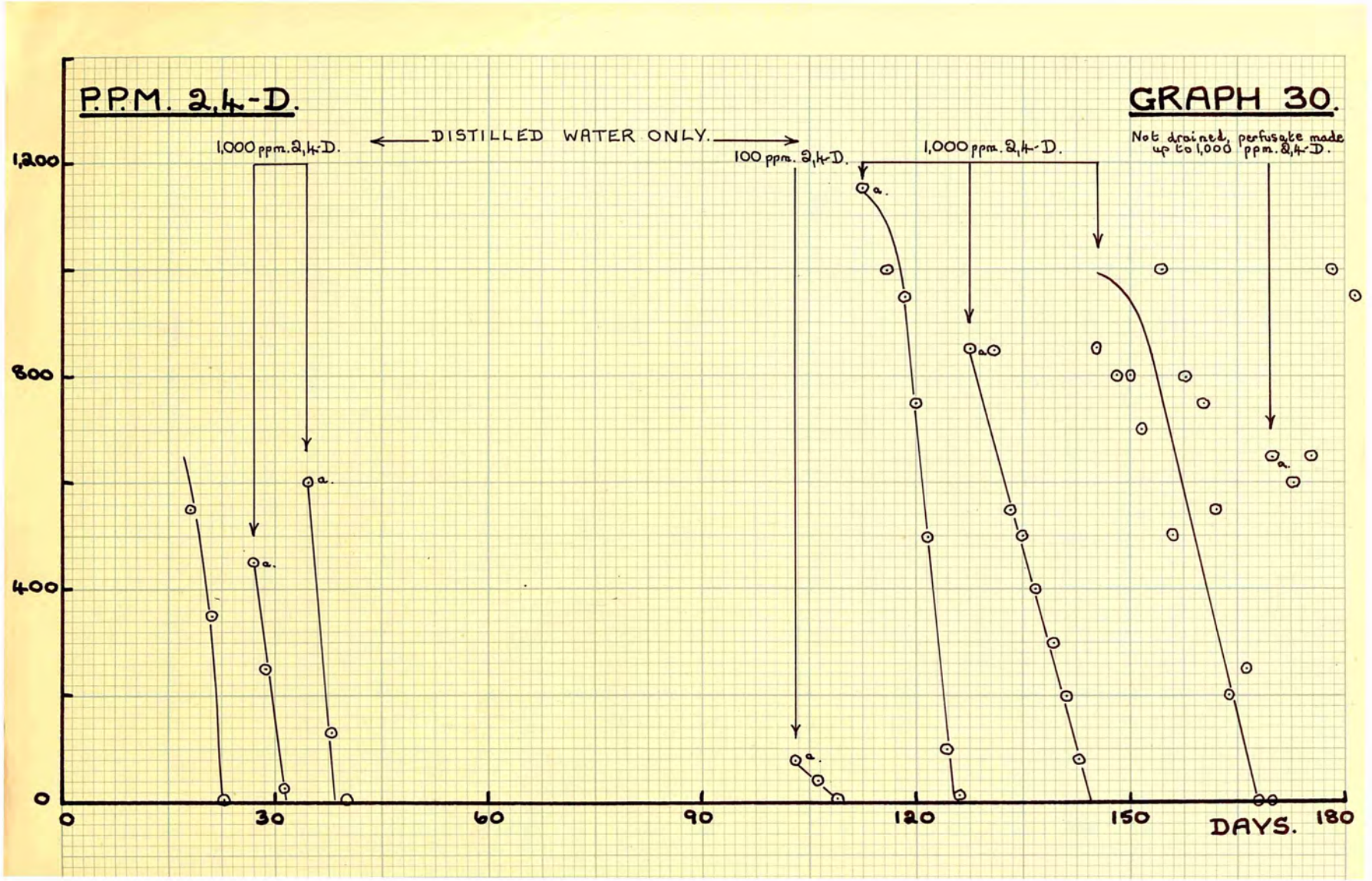
Only one attempt was made to enrich soil from another source. The soil was very different, being rich and heavy from a well cultivated allotment. The lag with 1,000 ppm. 2,4-D was of the same order as before. Circumstances prevented more accurate determination of the lag other than in the 20 day region (Graph and Table 38,).

Though enrichment behaviour was consistent at both high and low concentrations, it was only at low concentrations that subsequent behaviour was reliable and predictable with high rates of breakdown. At the higher concentrations breakdown rates were very variable with, sometimes, apparent loss of adaptation. This may have been due to the inability of the assay method to detect very slow changes in herbicide concentration. The erratic behaviour at high concentrations was observed in perfusers which were drained before

recharging as well as in those in which the 2,4-D concentration was made up by adding concentrated stock solution without draining. Thus, though the effect may have been due to toxic breakdown products, these could only have been intermediates and not accumulated end products.

Stability and Duration of 2,4-D adaptation.

Adaptation to 2,4-D proved to be a very stable and durable phenomenon. One perfuser in particular proved to be an outstanding illustration of this (Table 29,). After direct adaptation at 1,000 ppm., the perfuser was maintained in activity for 407 days by repeatedly recharging at 1,000 ppm., with or without draining. A wide variation in rates of breakdown was observed during this period. For the next two refills the perfusate concentration was lowered to 200 ppm. After 422 days the perfusate ceased to circulate so the perfusate and enriched soil were together placed in a plugged, sterile flask. From this point it functioned as a soil suspension. After each lot of 2,4-D had been completely broken down, sufficient stock 2,4-D solution and sterile distilled water was added to return the liquid to approximately 250 ml. of 100 ppm. The pattern of breakdown was fairly consistent for successive lots of 2,4-D but the rates were much slower than those experienced with perfusers. The rates showed a gradual improvement till, after about 890 days, the rate of breakdown was about half that in a normal perfuser. From this point the rates again decreased with successive refills though the suspension was still quite


P.P.M. 2,4-D.
GRAPH 30.
DISTILLED WATER ONLY.
1,000 ppm. 2,4-D.
100 ppm. 2,4-D.
1,000 ppm. 2,4-D.
Not drained, perfusate made up to 1,000 ppm. 2,4-D.
1,200
800
400
0
0
30
60
90
120
150
180
DAYS.

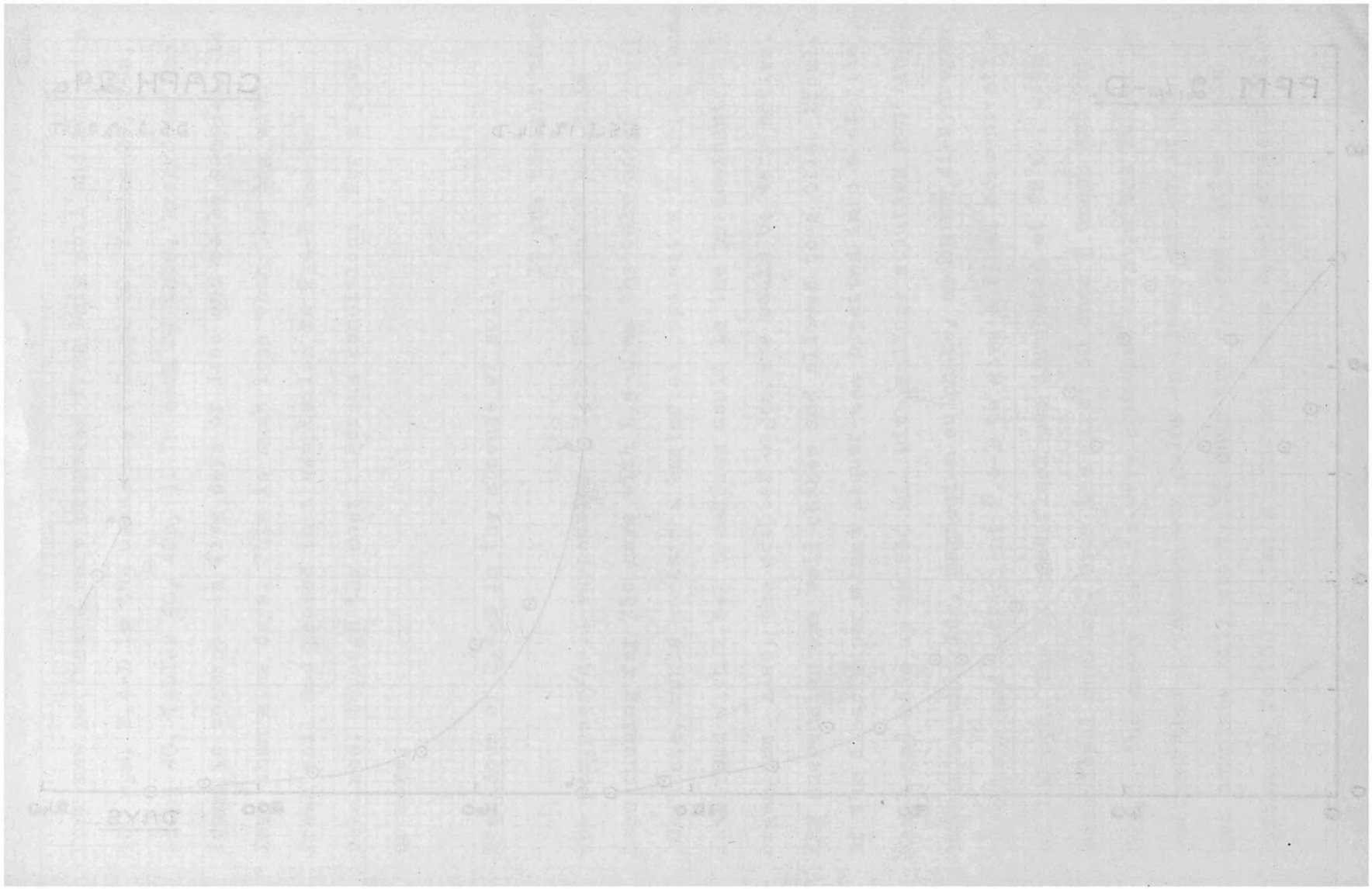
active when the last test was made, on the 971 st. day. The above perfuser/suspension showed the stability of the adapted population when the bacteria were regularly supplied with 2,4-D. It was also observed, following some cross-perfusion experiments, that adaptation to 2,4-D was retained during various periods of perfusion with other substrates (Graphs 41a, 42,42a,42c, Tables 41a, 42,42a,42b,42c,). Two other types of experiment were carried out to see if adaptation would be retained in the absence of any added substrate:

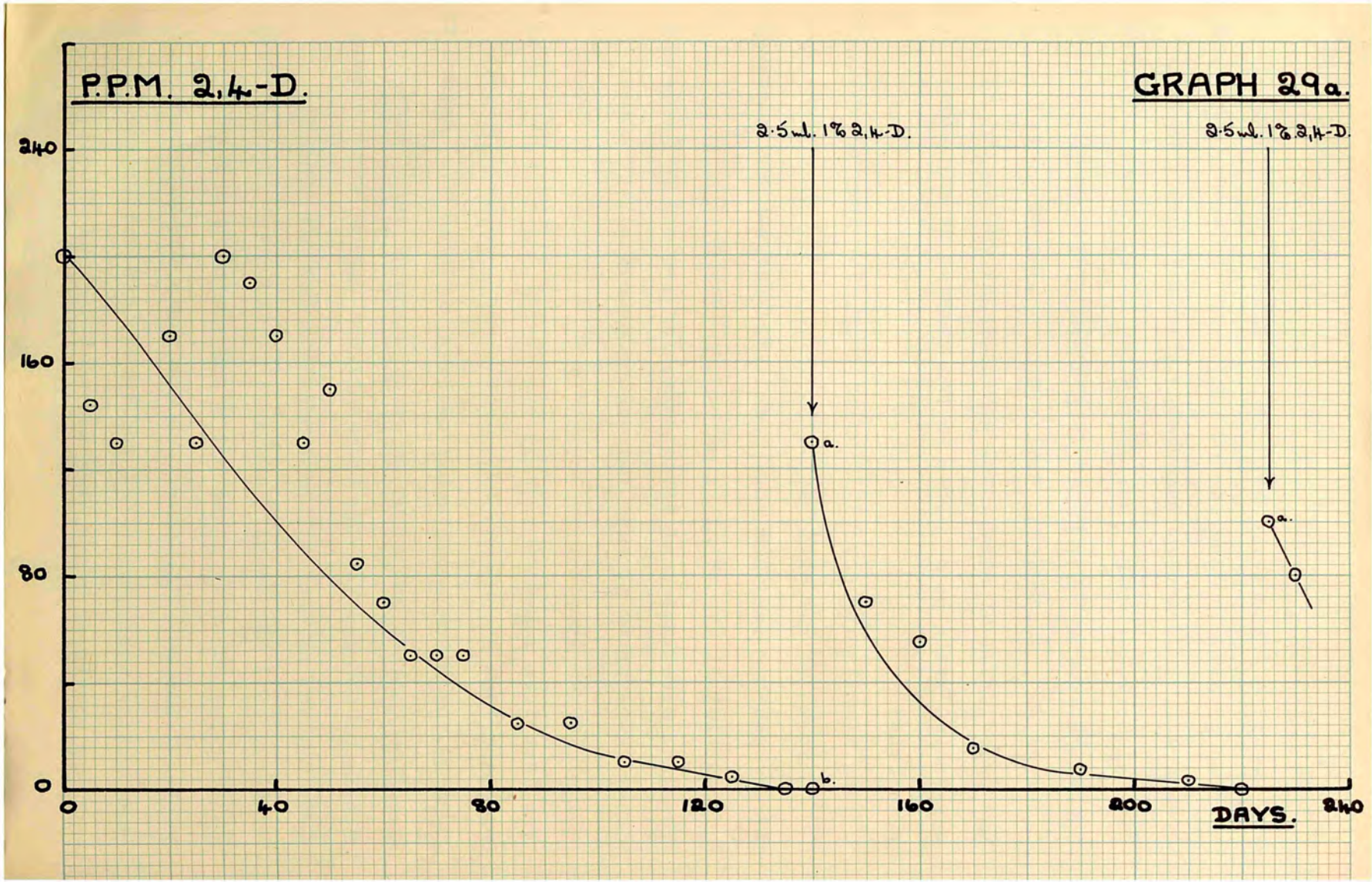
a). by circulating distilled water only, for long periods, through an enriched perfuser.

b). by storing enriched soil then, after drying and sieving, testing it for activity in new perfusers.

Type a). Circulation of water only, for various periods, was tried. Even after 60 days the soil remained active against 2,4-D though the rate of breakdown of a 100 ppm. solution was less than expected (Graph 30, Tables 30, 30b, 30c, 30d, 30e, 30f,).

Type b). When the large scale perfuser (Table 31.) ceased to function (mechanically) at an early stage of enrichment. 2,4-D, at 200 ppm. in the perfusate,was beeing broken down readily. The aluminium tube containing the 750 gms. of enriched soil was taken (stoppered by rubber bungs carrying small glass tubes to admit air) and stored in a cool cupboard for a little more than one year. The soil remained damp during this period. It was turned out and air-dried between sheets of paper then sieved to the usual sizes.

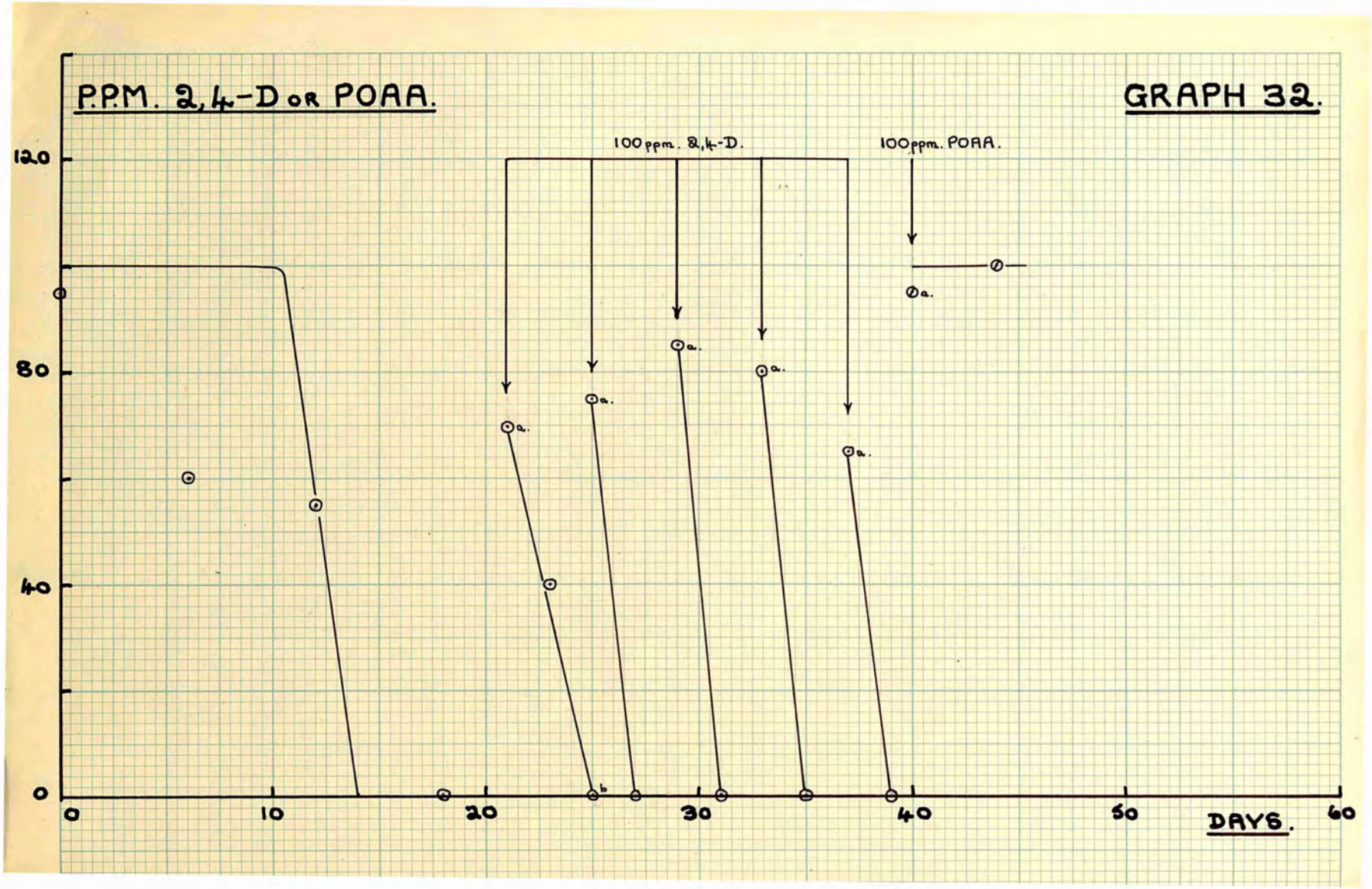


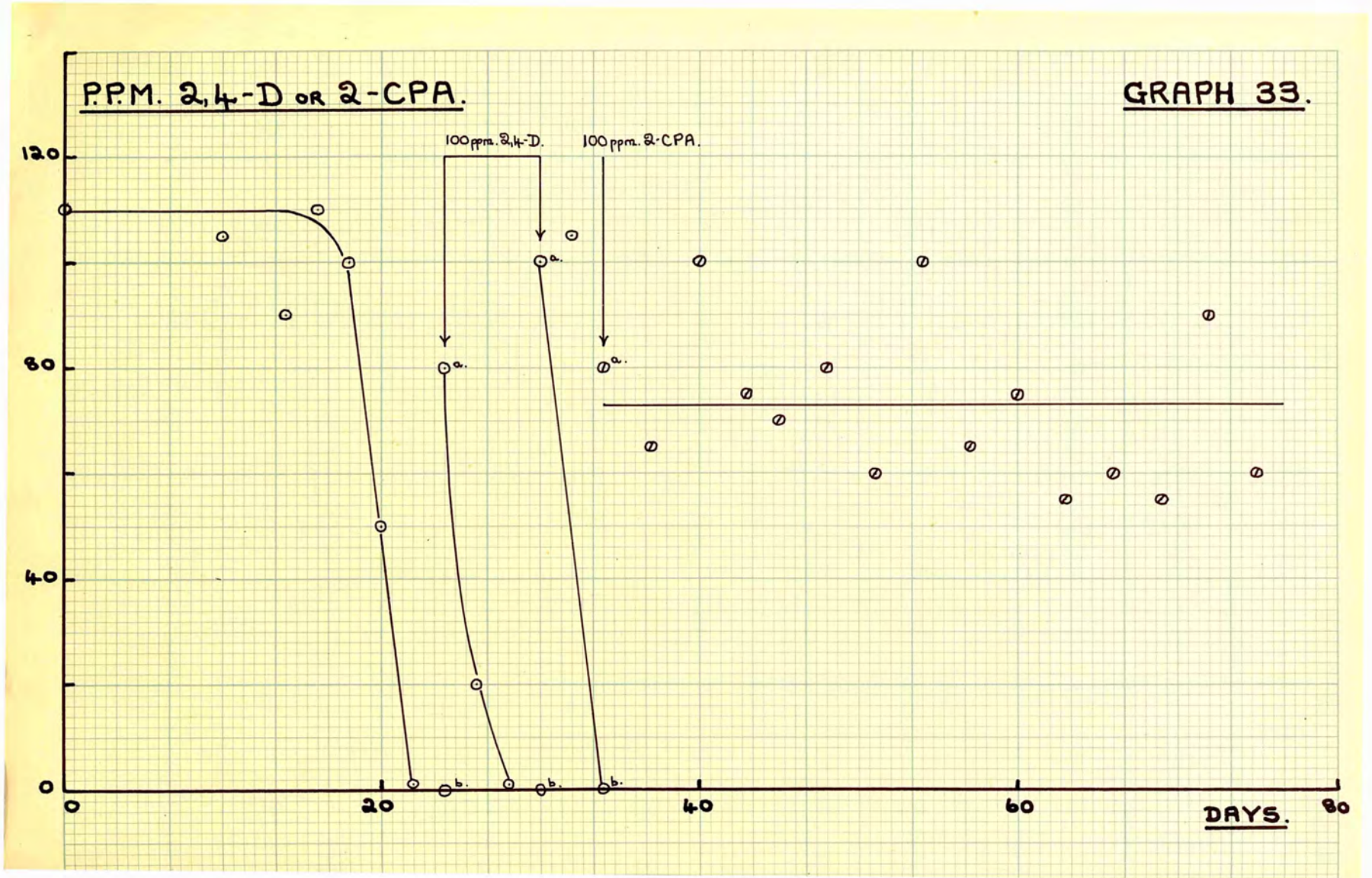

P.P.M. 2,4-D.
GRAPH 29a.
2·5 ml. 1% 2,4-D.
2·5 ml. 1% 2,4-D.
240
160
80
0
a.
a.
b.
0
40
80
120
160
200
240
DAYS.

Four new perfusers were prepared from this soil and run with 100 ppm. 2,4-D in the usual way (Graph 39, Tables 39, 39a, Graph 40, Tables 40, 40a,). In each of them, breakdown was found to commence in five days or less and to be complete in less than nine days. This is much less than the lag with fresh soil and proved that adaptation to 2,4-D can be retained, through the most rigorous conditions, for a year or more.

Breakdown of 2,4-D in the absence of soil.

It was thought that the perfuser/soil suspension (Table 29,), which had then been running for 756 days with 2,4-D as the only added substrate, would contain a bacterial population in which those concerned with 2,4-D breakdown would be the predominant organisms. Also, the adapted organisms would be very active. The suspension was well shaken and allowed to settle. 25 ml. of the clear supernatant liquor was pipetted into a sterile flask and made up to 250 ml. with culture solution containing potassium chloride, magnesium sulphate, ammonium di-hydrogen phosphate and sufficient 2,4-D to give a final concentration of 100 ppm. The plugged flask was incubated at 28°C., with occasional shaking. Over the first 50 days (Graph and Table 29a,), the assay results were somewhat erratic but it can be assumed that breakdown was going on slowly though it was not complete till about 130 days had elapsed. After again making up to 250 ml. at a somewhat lower 2,4-D concentration


P.P.M. 2,4-D OR POAA.
GRAPH 32.
100 ppm. 2,4-D.
100 ppm. POAA.
120
80
40
0
0
10
20
30
40
50
60
DAYS.
a.
b


P.P.M. 2,4-D OR 2-CPA.
GRAPH 33.
100 ppm. 2,4-D.
100 ppm. 2-CPA.
120
80
40
0
0
20
40
60
80
DAYS.
a.
b.

breakdown continued at more or less the same rate. A further recharge produced the same result.

Another bacterial suspension was prepared (Table 29b,) by adding 50 ml. of the above culture to a sterile solution of 2,4-D (100 ppm. as ammonium salt), plugging the flask and again incubating at 28°C. Slow breakdown occurred and was complete in about 40 days. After recharging there was no evidence of breakdown in a further 25 days.

2,4-D followed by phenoxyacetic acid (POAA).

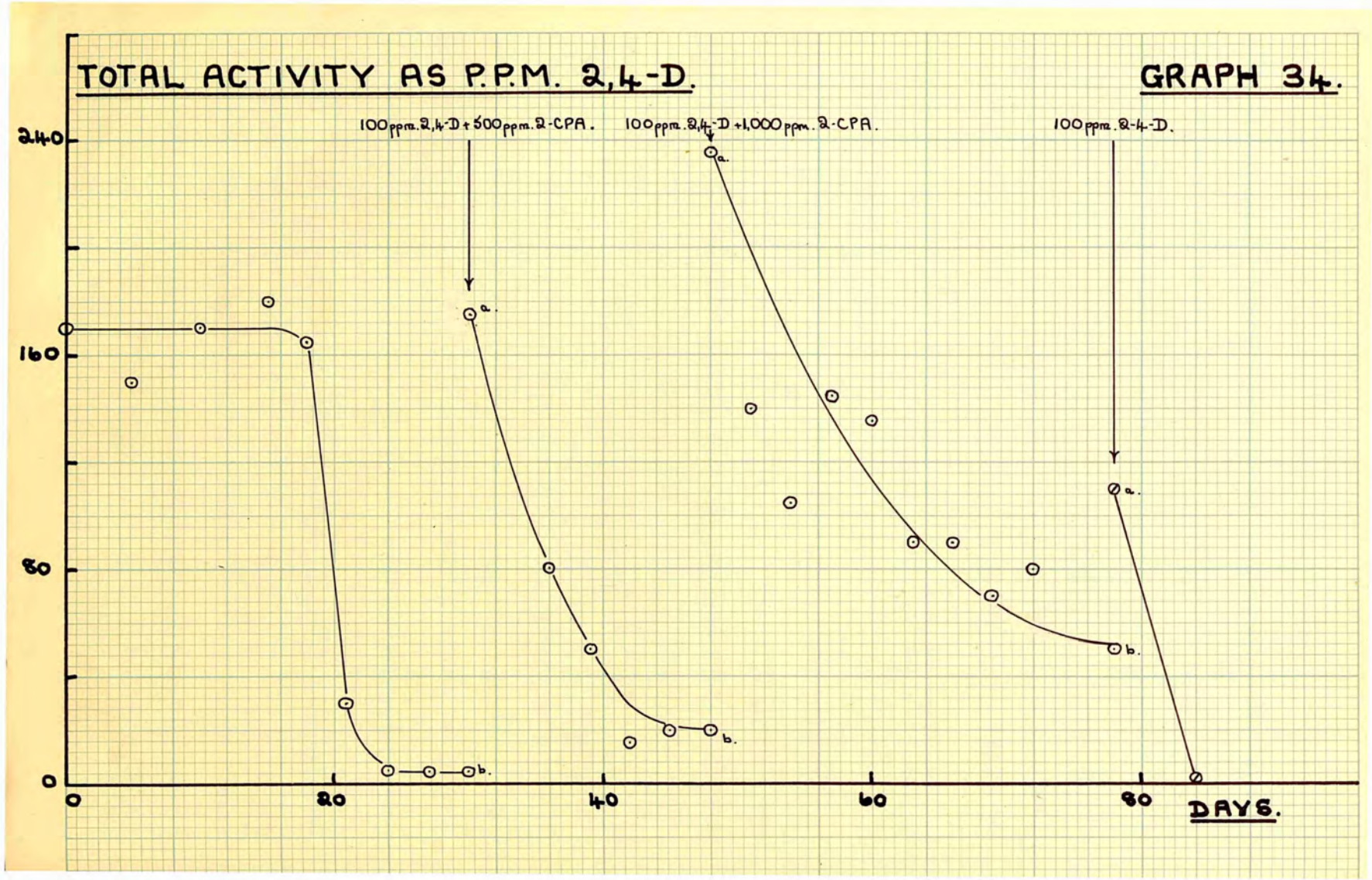
A 2,4-D perfuser was brought to a high state of activity through several changes of perfusate at 100 ppm. (Graph and Table 32,). It was then drained and refilled at 100 ppm. with 250 ml. of POAA solution. It was only possible to follow the perfusion for a further four days. No measureable breakdown occurred in this time which was more than long enough for complete decomposition if cross-adaptation had existed.

2,4-D followed by 2-chlorophenoxyacetic acid (2-CPA).

100 ppm.

2-CPA was added to each of four perfusers which had been enriched to 100 ppm. 2,4-D. Two were followed for a further 40 days and the other two for a further 70 days. In all four cases there was no evidence of 2-CPA breakdown.(Graph 33, Tables 33, 33a, 33b, 33c,).

While running on 2-CPA, the perfusates remained practically colourless whereas, in common with other perfusers actively


TOTAL ACTIVITY AS P.P.M. 2,4-D.
GRAPH 34.
100 ppm. 2,4-D + 500 ppm. 2-CPA.
100 ppm. 2,4-D + 1,000 ppm. 2-CPA.
100 ppm. 2-4-D.
240
160
80
0
0
20
40
60
80
DAYS.
a.
b.

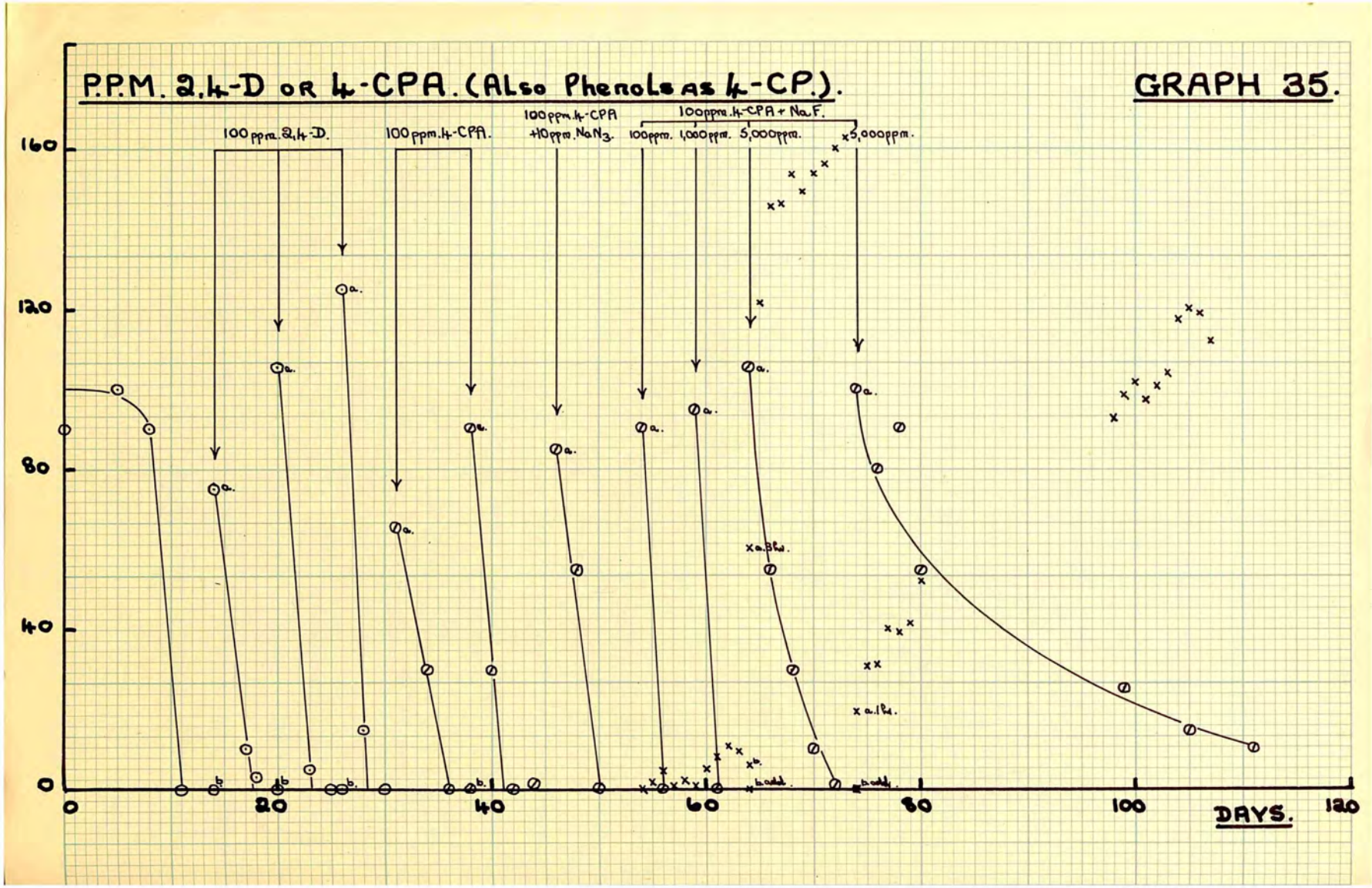
breaking down added herbicide, the perfusate had been slightly tinged with orange-pink while breaking down 2,4-D.

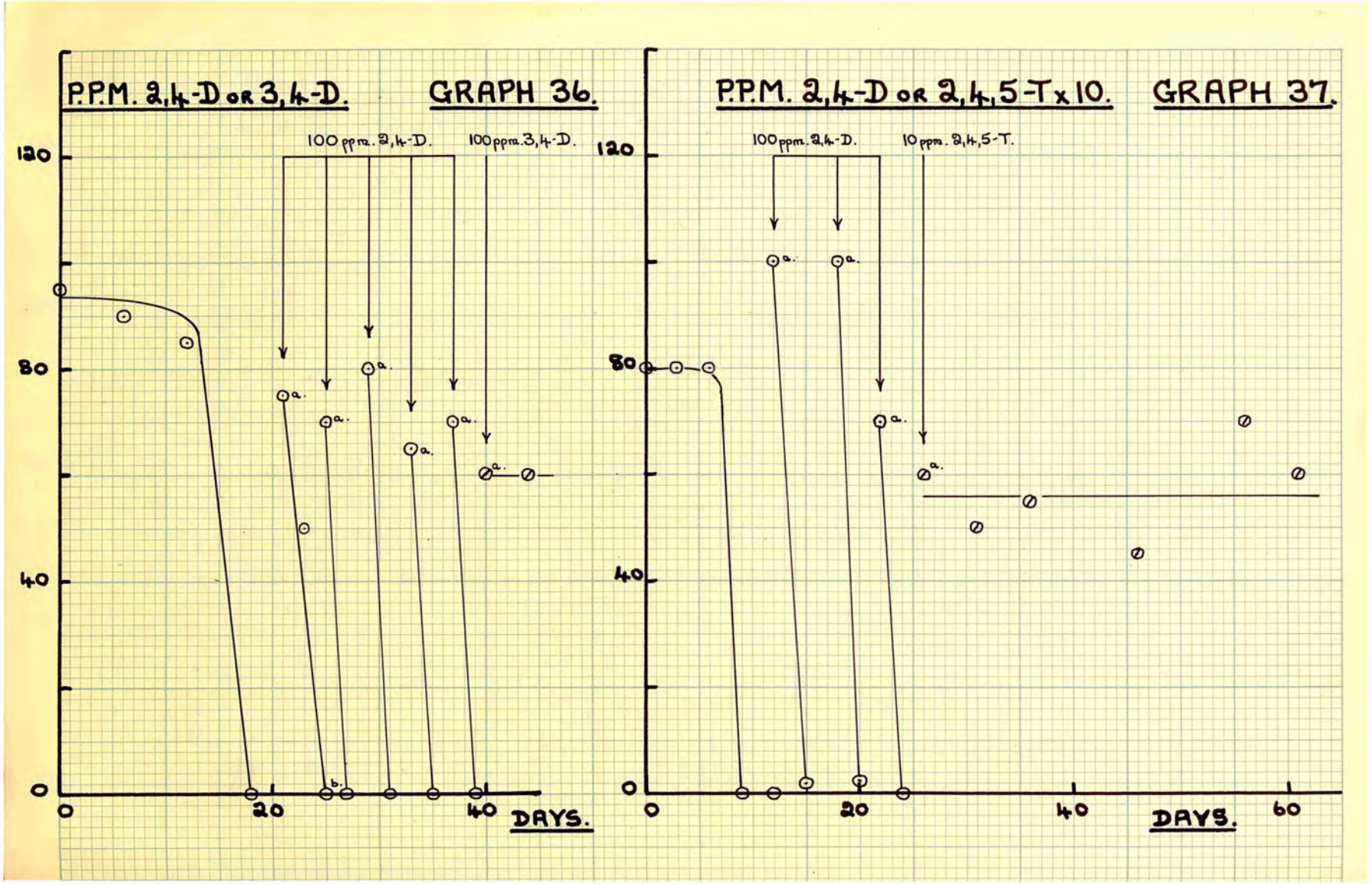
2,4-D/2-CPA mixtures.

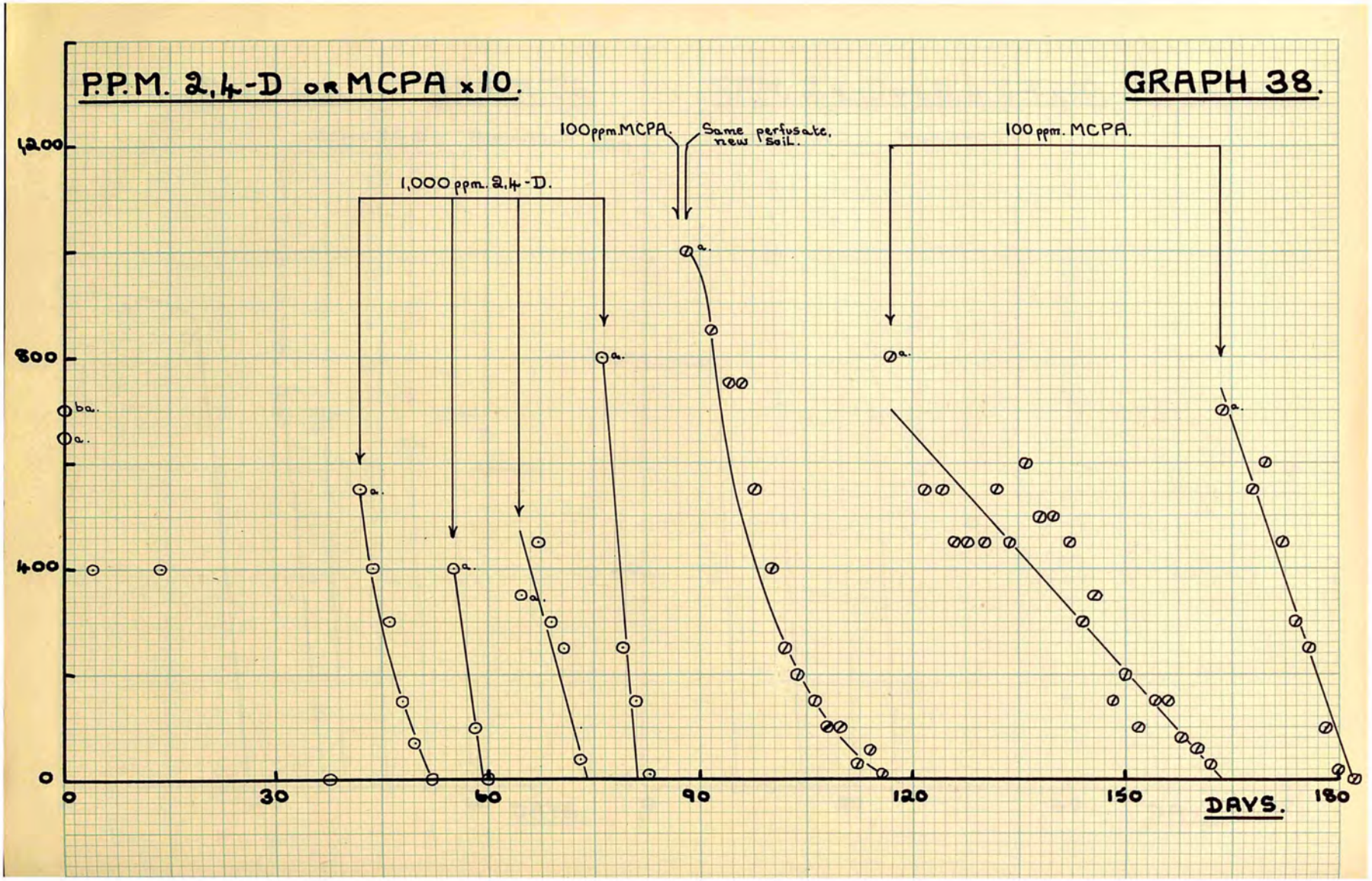
As with 4-CPA/2-CPA mixtures, 2,4-D/2-CPA mixtures caused a greater degree of inhibition in the assay test than could be attributed to a simple sumation of the activities of the components.

With a mixture containing 100 ppm. each of 2-CPA and 2,4-D, adaptation to the 2,4-D did occur in about 18 days, which is slightly longer than the normal 2,4-D lag. Once adaptation had occurred the 2,4-D was rapidly broken down and the residual activity of the perfusatevcould be attributed to the 100 ppm. 2-CPA only.(Graph 34, Tables 34 and 34a,). At this point the perfusers were drained and refilled with a mixture containing 100 ppm. 2,4-D and 500 ppm. 2-CPA. Again the 2,4-D was broken down though at a reduced rate. The perfusers were then drained and refilled with a mixture of 100 ppm. 2,4-D and 1,000 ppm. 2-CPA. Even this concentration of 2-CPA could not completely inhibit the breakdown reaction though it had a marked retarding effect on the rate of breakdown.

The inhibitory effect of 2-CPA cannot have been due to a permanent intoxication of the adapted bacteria, nor to a serious reduction in their numbers for, on draining and refilling the perfusers with 100 ppm. 2,4-D alone, a rapid rate of breakdown was achieved.


P.P.M. 2,4-D or 4-CPA. (Also Phenols as 4-CP.).
GRAPH 35.
100 ppm. 2,4-D.
100 ppm. 4-CPA.
100 ppm. 4-CPA +10 ppm. NaN3.
100 ppm. 4-CPA + Na F.
100 ppm.
1,000 ppm.
5,000 ppm.
5,000 ppm.
160
120
80
40
0
0
20
40
60
80
100
120
DAYS.


P.P.M. 2,4-D OR 3,4-D.
GRAPH 36.
100 ppm. 2,4-D.
100 ppm. 3,4-D.
120
80
40
0
0
20
40
DAYS.
a.
b.
P.P.M. 2,4-D OR 2,4,5-T x 10.
GRAPH 37.
100 ppm. 2,4-D.
10 ppm. 2,4,5-T.
120
80
40
0
0
20
40
60
DAYS.
a.


P.P.M. 2,4-D or MCPA x10.
GRAPH 38.
100ppm.MCPA.
Same perfusate, new soil.
100 ppm. MCPA.
1,000 ppm. 2,4-D.
1200
800
400
0
ba.
a.
0
30
60
90
120
150
180
DAYS.

2,4-D followed by 4-chlorophenoxyacetic acid (4-CPA).

In each of four attempts, 4-CPA was broken down rapidly and with no detectable lag when added at 100 ppm. to 2,4-D (100 ppm.) enriched perfusers (Graph 35, Tables 35, 35a, 35b, 35c,). Further additions of 4-CPA disappeared with equal rapidity.

2,4-D followed by 3,4-dichlorophenoxyacetic acid (3,4-D).

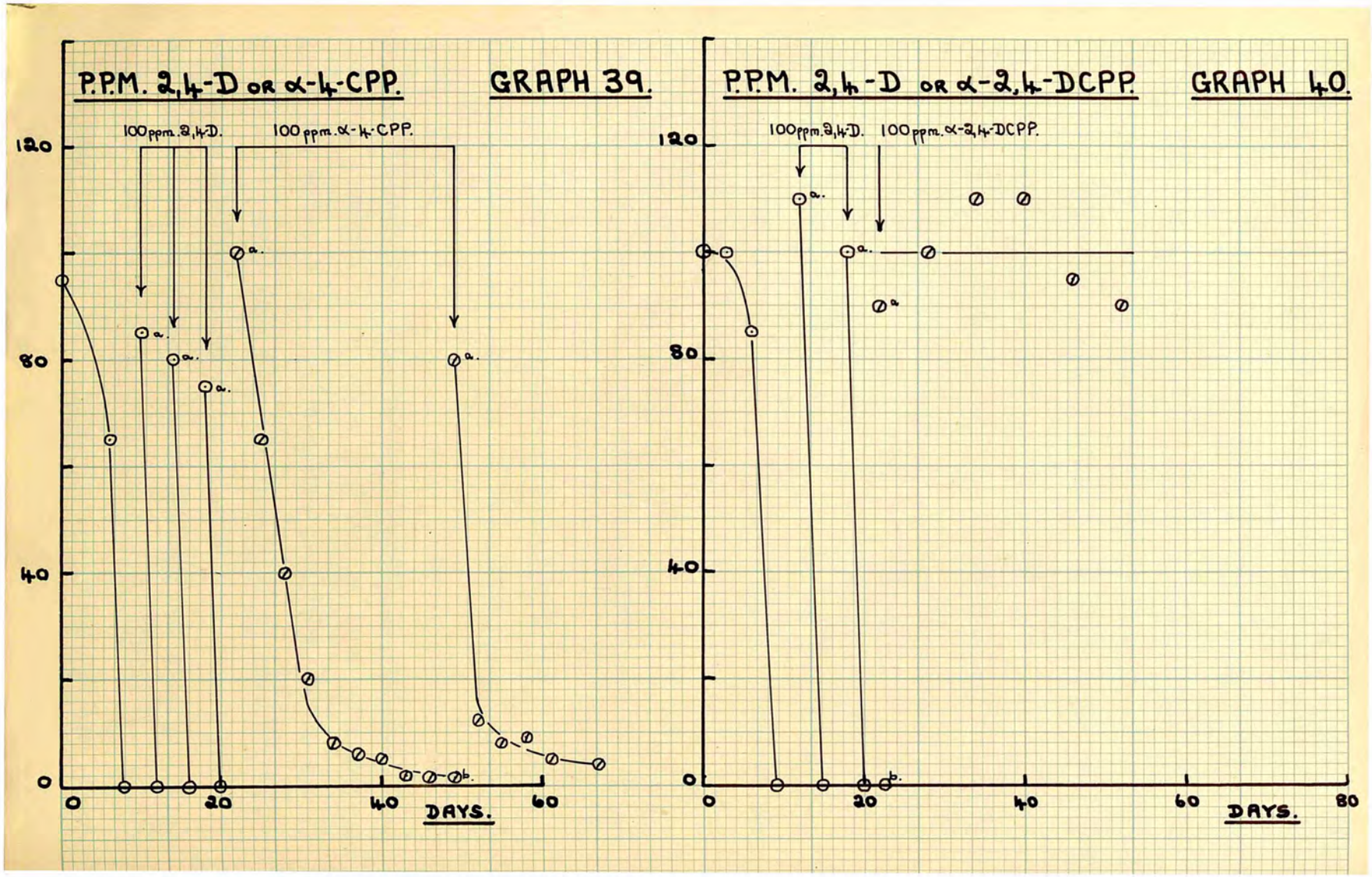
It was only possible to continue the perfusion for four days after draining a 2,4-D enriched perfuser and refilling it with 100 ppm. 3,4-D. No breakdown was detected in this time which was probably long enough for complete disappearance of the 3,4-D if cross-adaptation had occurred (Graph and Table 36,).

2,4-D followed by 2,4,5- trichlorophenoxyacetic acid (2,4,5-T).

10 ppm. 2,4,5-T showed no signs of breakdown during 35 days perfusion in 2,4-D enriched perfusers (100 ppm.) (Graph 37, Tables 37, 37a,).

2,4-D followed by 4-chloro,2-methylphenoxyacetic acid (MCPA).

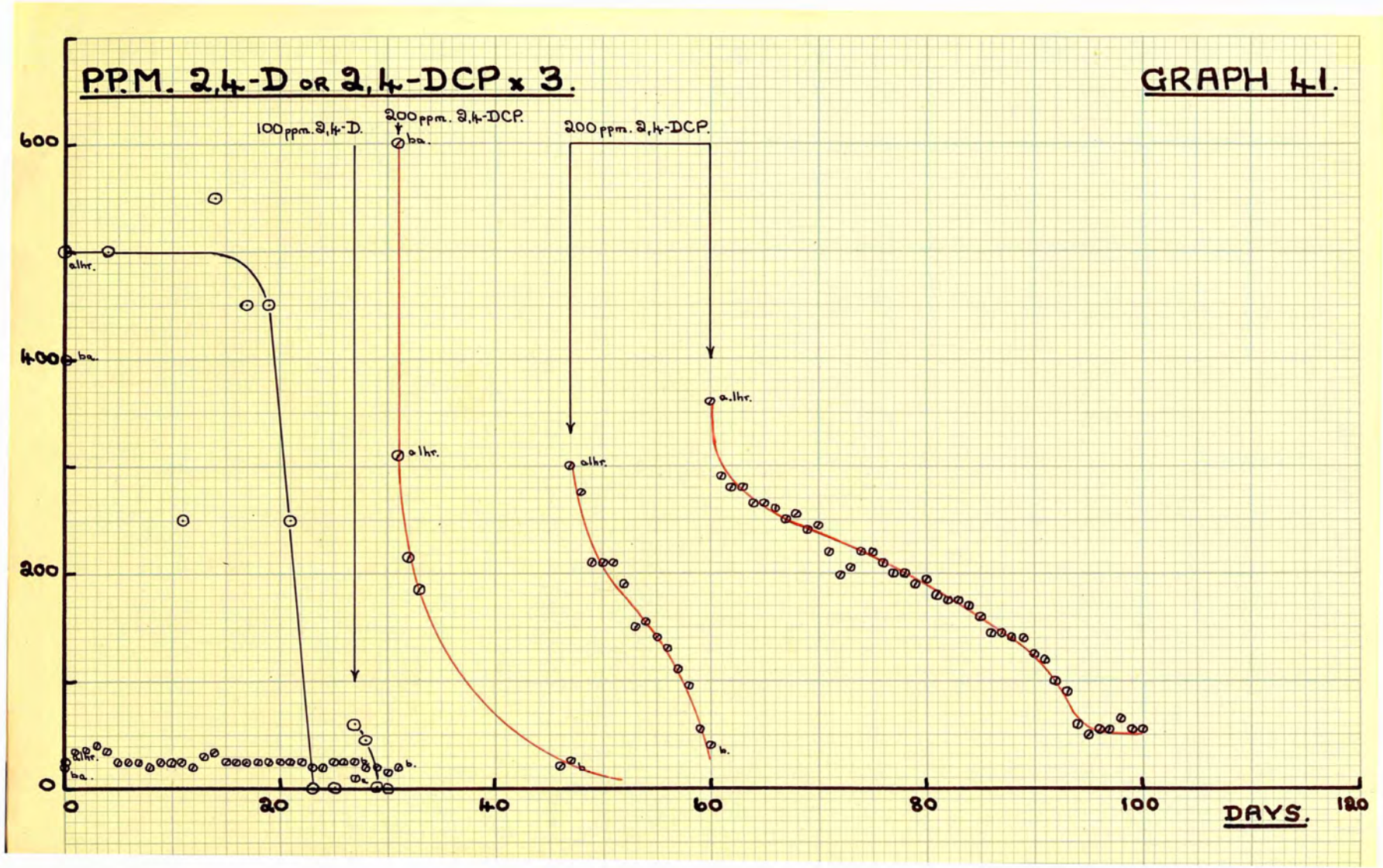
Breakdown of MCPA at 100 ppm. appeared to commence as soon as it was added to a 2,4-D enriched perfuser (Graph and Table 38,). The rate of breakdown was slow when compared with that of a straightforward MCPA enriched perfuser. On draining and refilling with 100 ppm. MCPA, the rate of breakdown was even slower but increased on refilling again. From the results it may be assumed that adaptation to 2,4-D also involves


P.P.M. 2,4-D OR α-4-CPP.
GRAPH 39.
100 ppm. 2,4-D.
100 ppm. α-4-CPP.
120
80
40
0
0
20
40
60
DAYS.
P.P.M. 2,4-D OR α-2,4-DCPP.
GRAPH 40.
100 ppm. 2,4-D.
100 ppm. α-2,4-DCPP.
120
80
40
0
0
20
40
60
80
DAYS.

adaptation to MCPA. The breakdown rates should not be strictly compared with those for MCPA in other perfusers for the soil initially enriched to 2,4-D was from a different source and, because of perfusion difficulties, the adaptation had to be transferred to fresh soil at the same time as the switch to MCPA. Table 38a shows the results obtained with the usual soil but higher 2,4-D and MCPA concentrations.

2,4-D followed by ∝-4-chlorophenoxyacetic acid (∝-4-CPP). Rapid breakdown of ∝-4-CPP commenced as soon as it was added, at 100 ppm., to perfusers enriched to 100 ppm. 2,4-D. (Graph 39, Tables 39, 39a,). The rate of breakdown was slightly higher after draining and refilling. In both perfusers breakdown appeared not to go to completion but tended towards a low residual concentration. This is consistent with the theory that only one of the optical isomers is broken down and that it is this one which contributes the major part of the physiological activity of the racemic mixture.

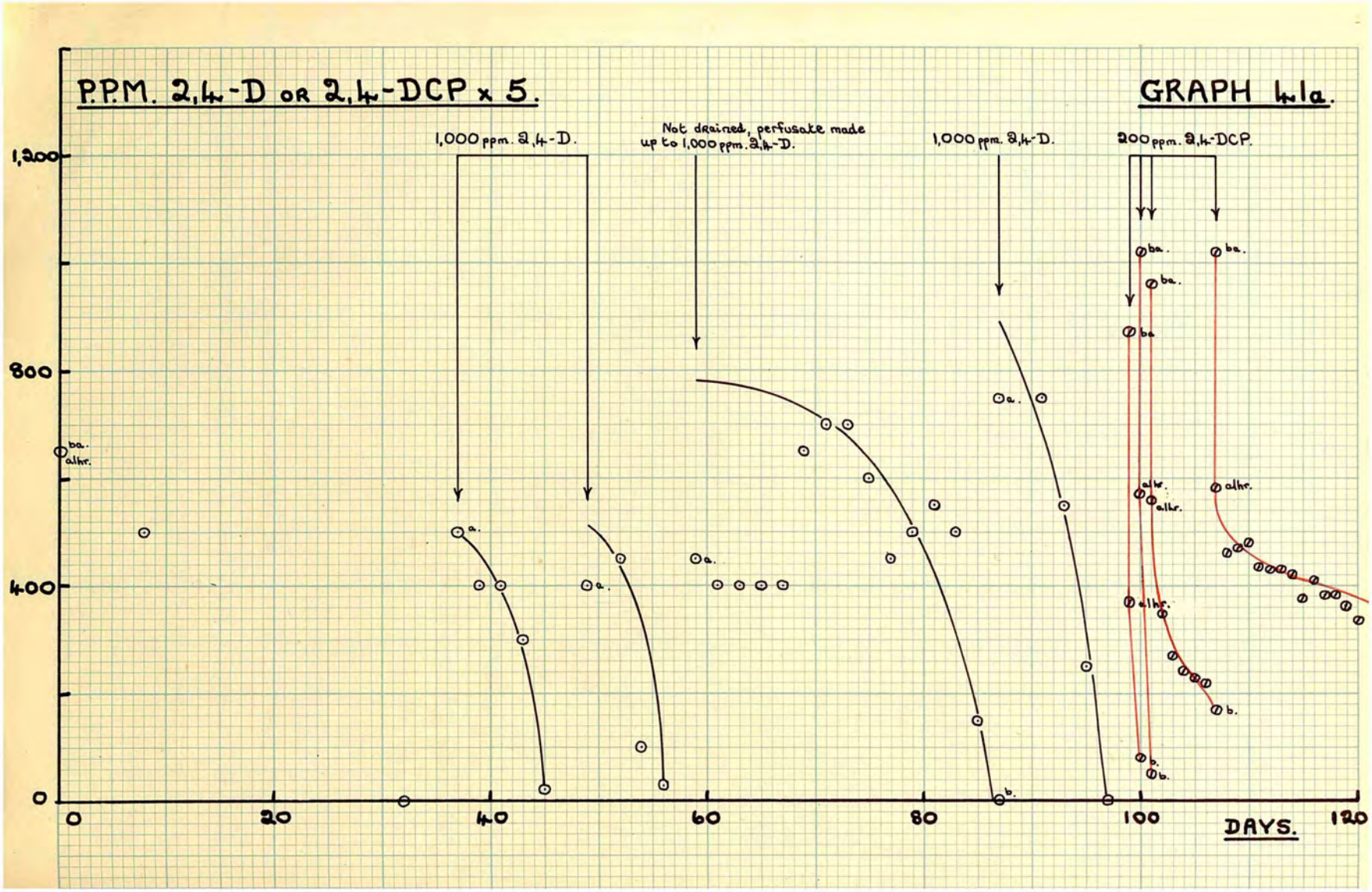
2,4-D followed by ∝-2,4-dichlorophenoxypropionic acid (∝-2,4-DCPP). There was no evidence of breakdown in 30 days when ∝-2,4-DCPP was added at 100 ppm. to a perfuser enriched to 100 ppm. 2,4-D (Graph 40, Tables 40, 40a,). This apparently anomolous behaviour does not preclude the possibility, though it is most unlikely, that in the case of ∝-2,4-DCPP the 2,4-D adapted organisms again

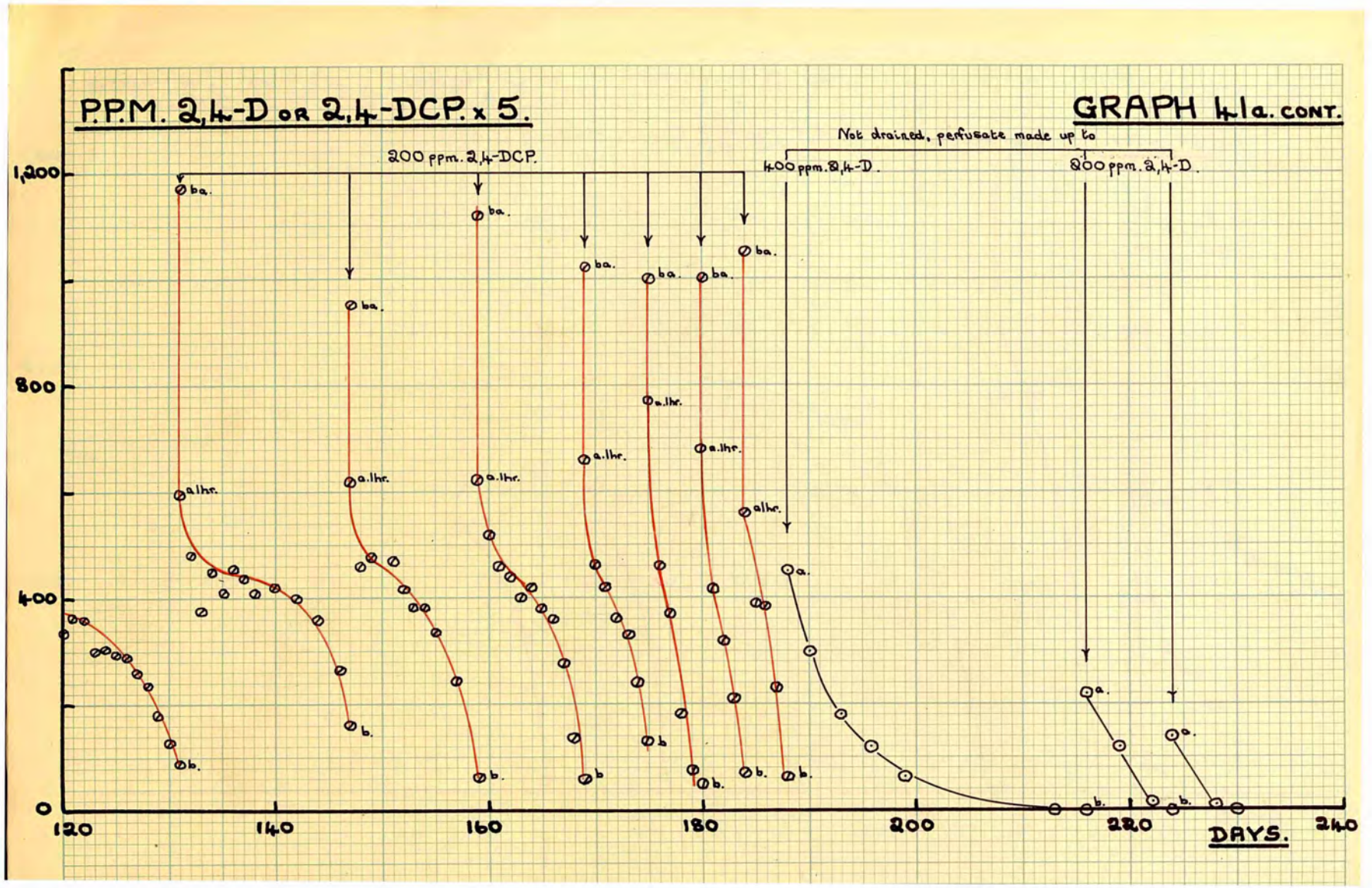

P.P.M. 2,4-D OR 2,4-DCP x 3.
GRAPH 41.
100 ppm. 2,4-D.
200 ppm. 2,4-DCP.
200 ppm. 2,4-DCP.
a1hr.
ba.
600
400
200
0
0
20
40
60
80
100
120
DAYS.

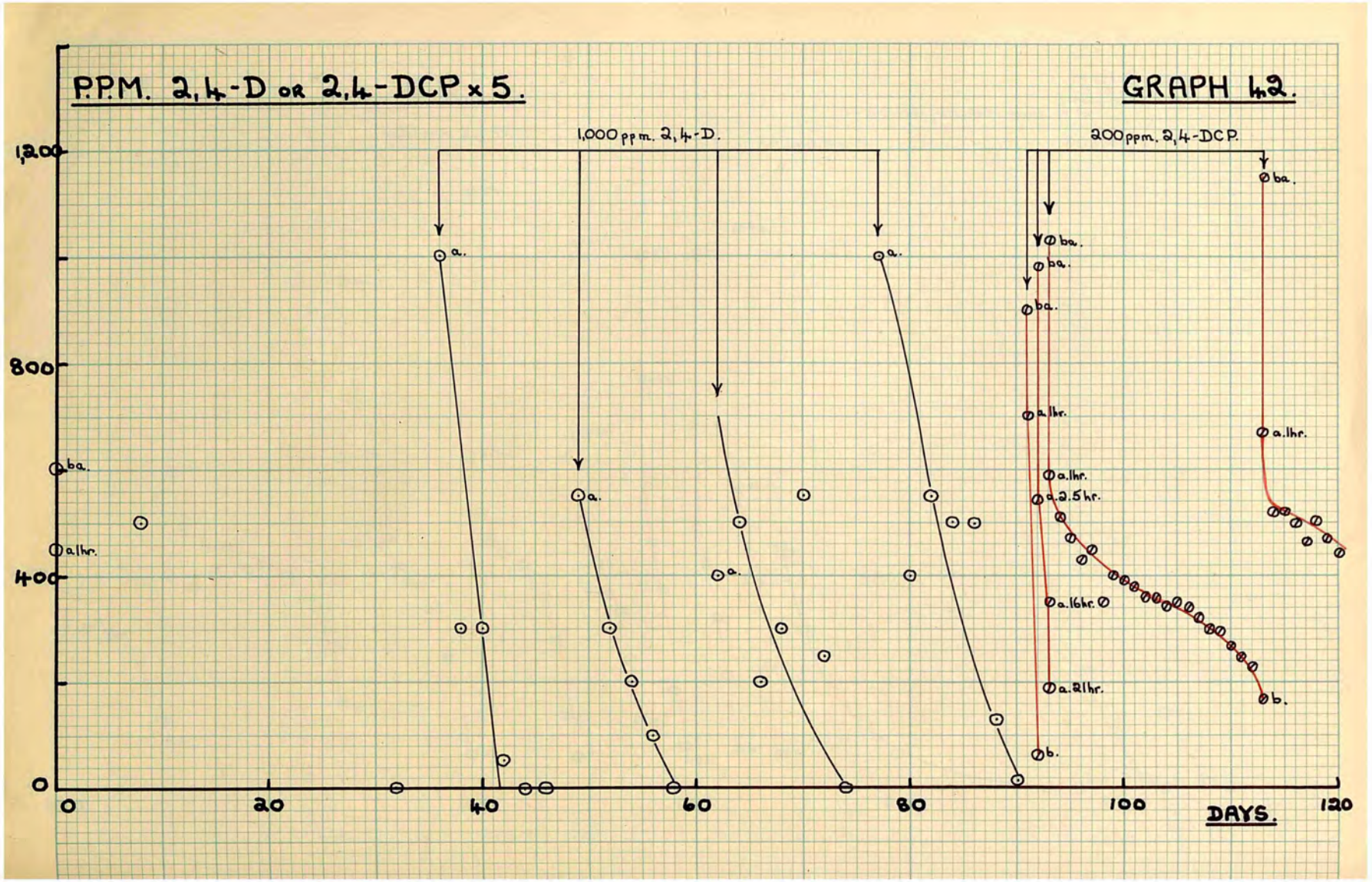
responded differently to the two optical isomers but that in this case it was the least physiologically active which was decomposed. The change in total activity would then probably be indetectable by simple assay.

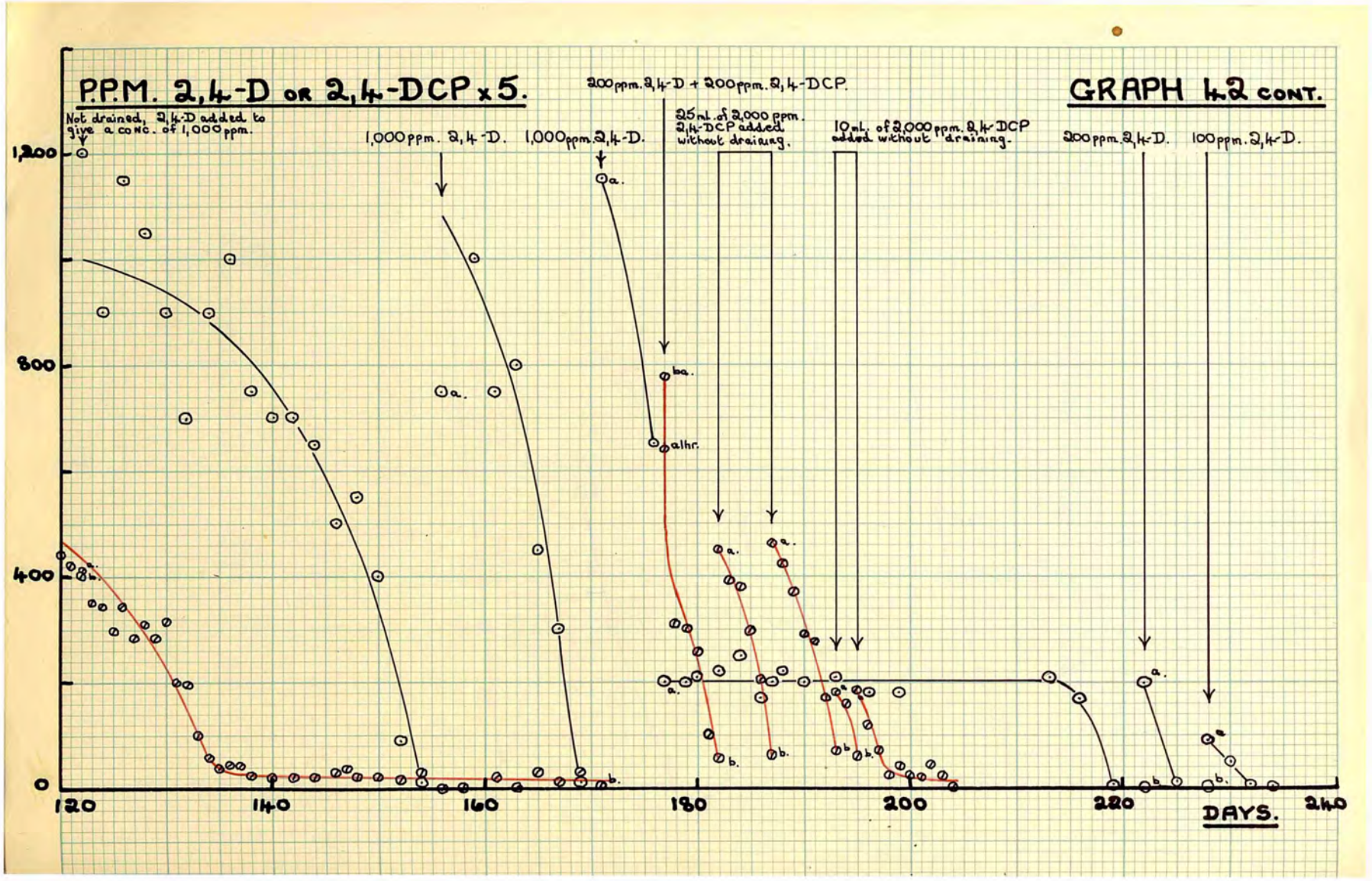
2,4-D followed by 2,4-dichlorphenol (2,4-DCP).

Because of the obvious possibility of 2,4-DCP being an intermediate in the 2,4-D degradation chain, the results of adding 2,4-DCP to enriched 2,4-D perfusers were studied several times with various modifications (Graphs 41, 41a, 42, Tables 30d, 30e, 30f, 38a, 41, 41a, 41b, 41c, 41d, 41e, 42, 42f,).
Except for two anomalous perfusers (Tables 30d, 41b,) the same general behaviour pattern was observed whenever an enriched 2,4-D perfuser was drained and refilled with 2,4-DCP solution. The 2,4-DCP disappeared very rapidly from the perfusate, even faster than the prior charge of 2,4-D. Sometimes a further recharge of 2,4-DCP disappeared with equal rapidity (Graphs 41a, 42, Tables 30e, 41a, 42,) but usually, on adding this second charge of 2,4-DCP, the perfuser appeared to lose its ability to deal with the compound. It is unlikely that the loss of activity was due to loss of adapted bacteria on draining the system for, the effect was not observed at later stages of the same perfusers, nor in directly enriched 2,4-DCP perfusers. The inhibition also occurred in perfusers which were recharged without draining.
The perfusers appeared to enter a new lag phase in which the


P.P.M. 2,4-D OR 2,4-DCP x 5.
GRAPH 4.1a.
1,000 ppm. 2,4-D.
Not drained, perfusate made up to 1,000 ppm. 2,4-D.
1,000 ppm. 2,4-D.
200 ppm. 2,4-DCP.
1,200
800
400
0
ba.
a1hr.
a.
b.
0
20
40
60
80
100
120
DAYS.


P.P.M. 2,4-D OR 2,4-DCP. x 5.
GRAPH 4.1a. CONT.
Not drained, perfusate made up to
200 ppm. 2,4-DCP.
400 ppm. 2,4-D.
200 ppm. 2,4-D.
1,200
800
400
0
120
140
160
180
200
220
240
DAYS.
ba.
a.1hr.
a.
b.


PPM. 2,4-D OR 2,4-DCP x 5.
GRAPH 42.
1,000 ppm. 2,4-D.
200 ppm. 2,4-DCP.
1,200
800
400
0
ba.
a.1hr.
a.
a.1hr.
a.2.5hr.
a.16hr.
a.21hr.
b.
0
20
40
60
80
100
120
DAYS.

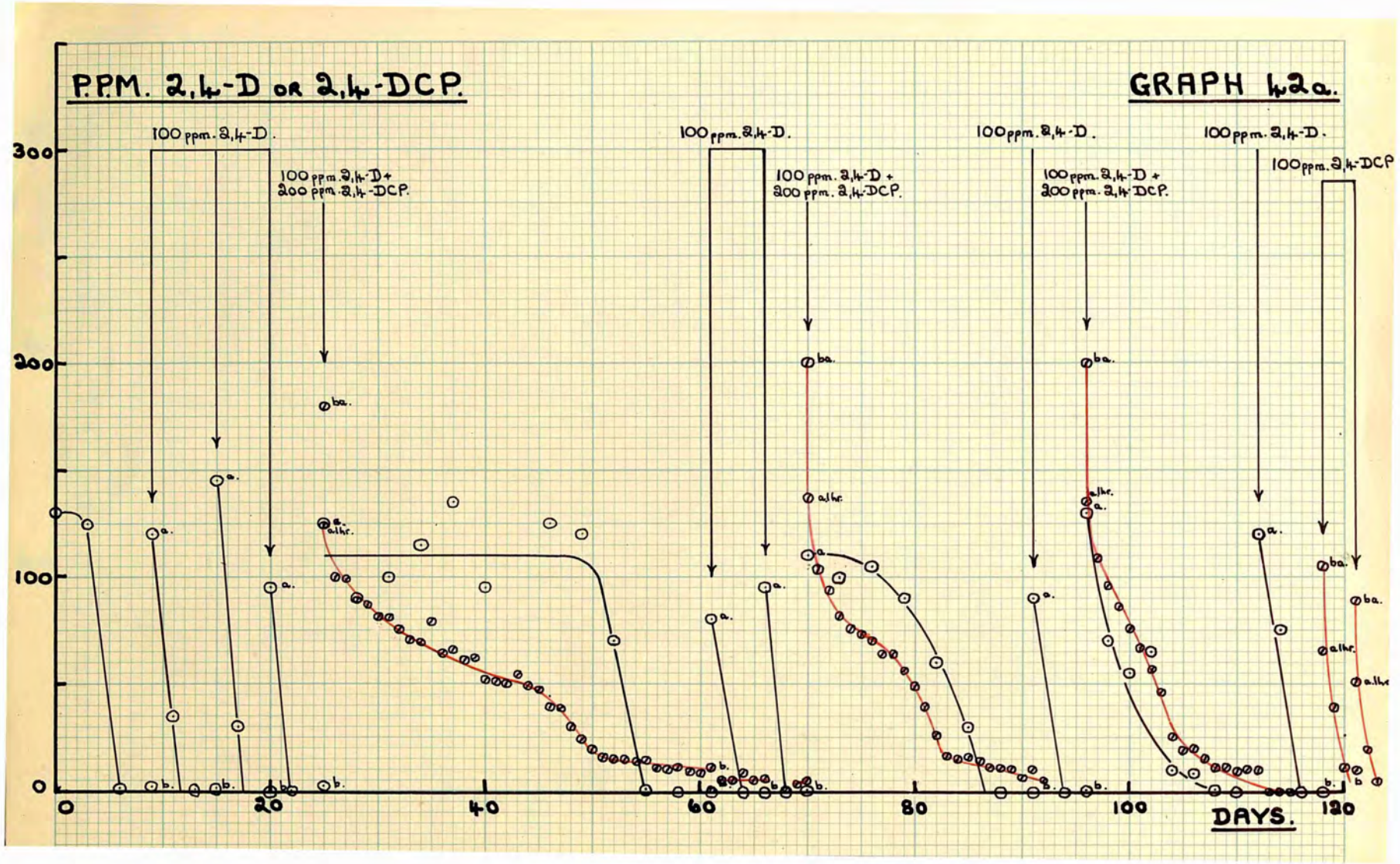

P.P.M. 2,4-D OR 2,4-DCP x 5.
GRAPH 4.2 CONT.
Not drained. 2,4-D added to give a conc. of 1,000 ppm.
1,000 ppm. 2,4-D.
1,000 ppm. 2,4-D.
200 ppm. 2,4-D + 200 ppm. 2,4-DCP.
25 ml. of 2,000 ppm. 2,4-DCP added without draining.
10 ml. of 2,000 ppm. 2,4-DCP added without draining.
200 ppm. 2,4-D.
100 ppm. 2,4-D.
1,200
800
400
0
120
140
160
180
200
220
240
DAYS.

2,4-DCP disappearing slowly at a rate probably no greater than the evaporation rate for each perfuser (cf. Graph 41 where the system had been poisoned with 0.01% sodium azide and phenol disappearance was probably by evaporation only). The same behaviour pattern prevailed in perfusers enriched to 100 or 1,000 ppm. 2,4-D and when the added 2,4-DCP was at 50, 100, 200 or 400 ppm.

After a period of 20 to 40 days, during which the 2,4-DCP was disappearing slowly, a new enrichment seemed to occurr and the 2,4-DCP disappeared with increasing rapidity. The rate of 2,4-DCP breakdown further increased with successive draining and recharging of the perfusers. Activity was maintained thereafter with no further sign of de-adaptation. In only one case was the perfuser returned to 2,4-D after the 2,4-DCP lag and re-adaptation (Graph 41a,). The adaptation to 2,4-D had been retained.

2,4-D followed by a mixture of 2,4-D and 2,4-DCP.

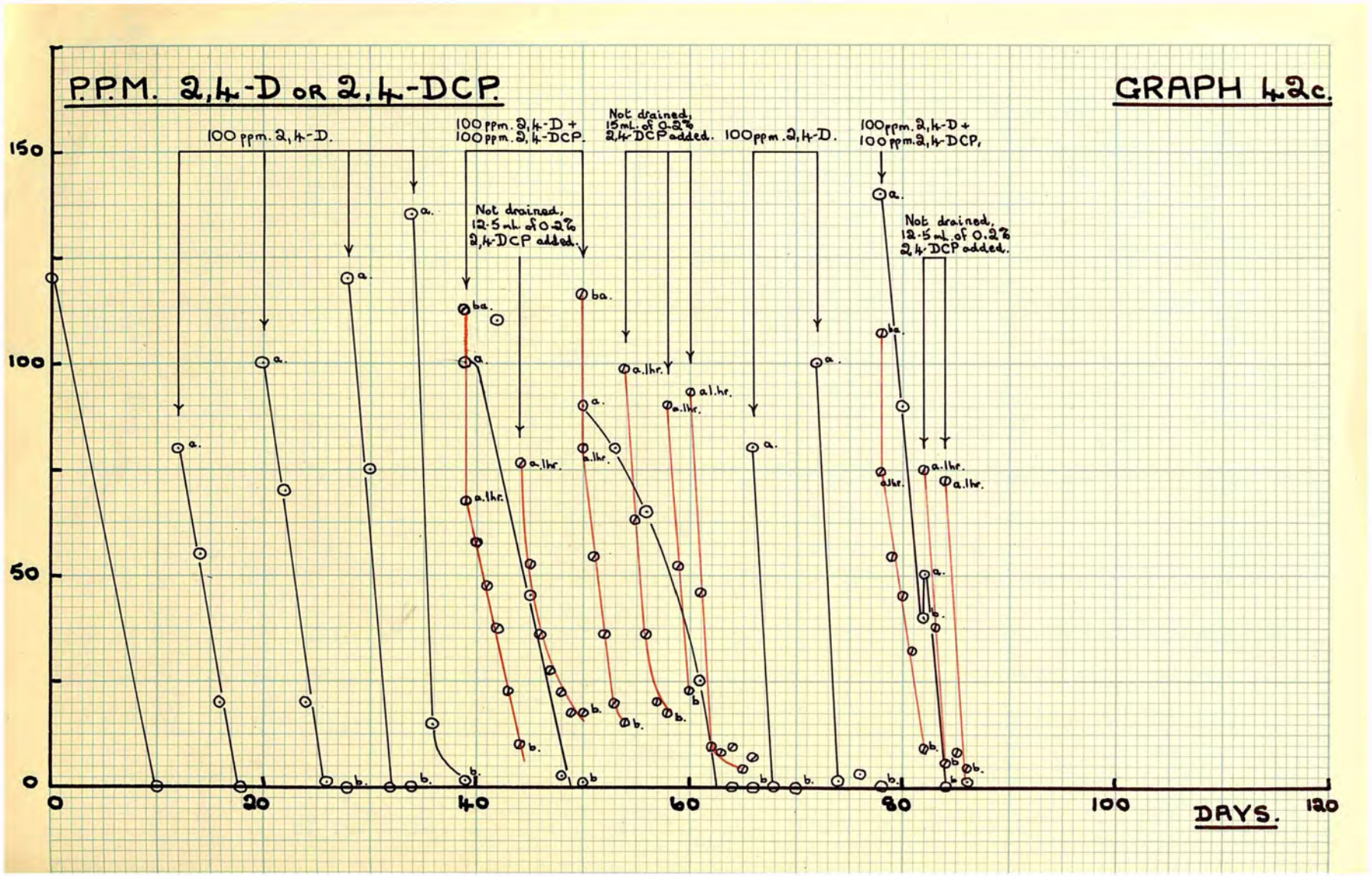
With the first of these perfusers, enriched to 1,000 ppm. 2,4-D, 2,4-DCP was first added at 200 ppm. (Graph and Table 42,). The normal course was followed with the phenol disappearing rapidly after the first two refills but going into a lag phase, with slow breakdown, on refilling a third time. Draining and again refilling with 2,4-DCP did not materialy alter the breakdown rate. During this second period, sufficient 2,4-D was added without draining to produce a concentration of 1,000 ppm. in the perfusate. The 2,4-DCP


P.P.M. 2,4-D or 2,4-DCP.
GRAPH 42a.
100 ppm. 2,4-D.
100 ppm. 2,4-D + 200 ppm. 2,4-DCP.
100 ppm. 2,4-D.
100 ppm. 2,4-D + 200 ppm. 2,4-DCP.
100 ppm. 2,4-D.
100 ppm. 2,4-D + 200 ppm. 2,4-DCP.
100 ppm. 2,4-D.
100 ppm. 2,4-DCP
300
200
100
0
0
20
40
60
80
100
120
DAYS.

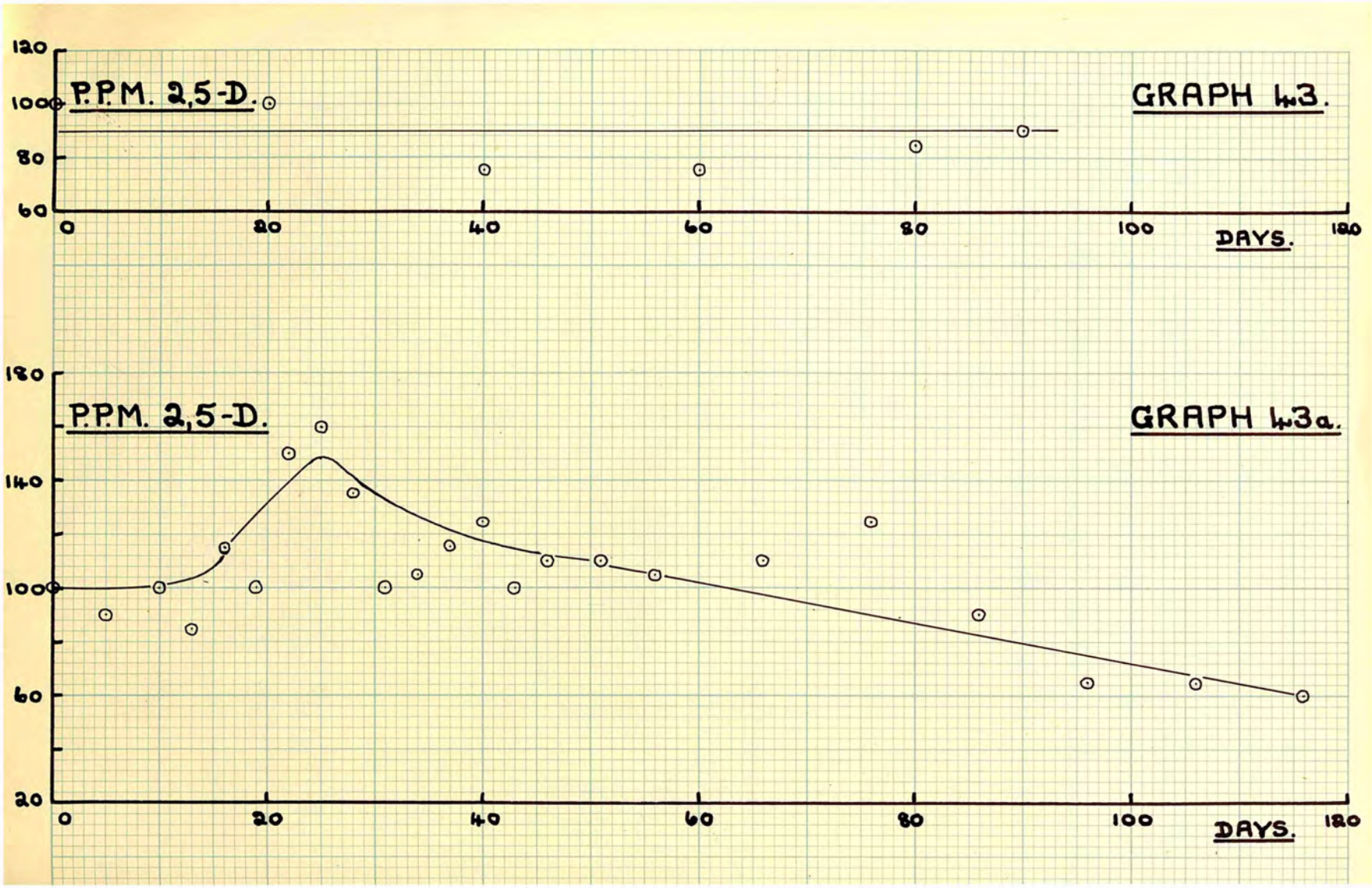
breakdown rate was not appreciably altered and at first the 2,4-D disappearance followed a slow parallel course. When virtually all the 2,4-DCP had gone, the rate of 2,4-D breakdown rose sharply. After draining, two further charges of 2,4-D alone also disappeared quickly. The perfuser was once more filled with 2,4-D/2,4-DCP mixture and the 2,4-DCP replenished, as required, without draining the perfuser. The 2,4-DCP disappeared repeatedly at a high rate and eventually was not replenished. The 2,4-D was not broken down till long after the last lot of 2,4-DCP had gone.

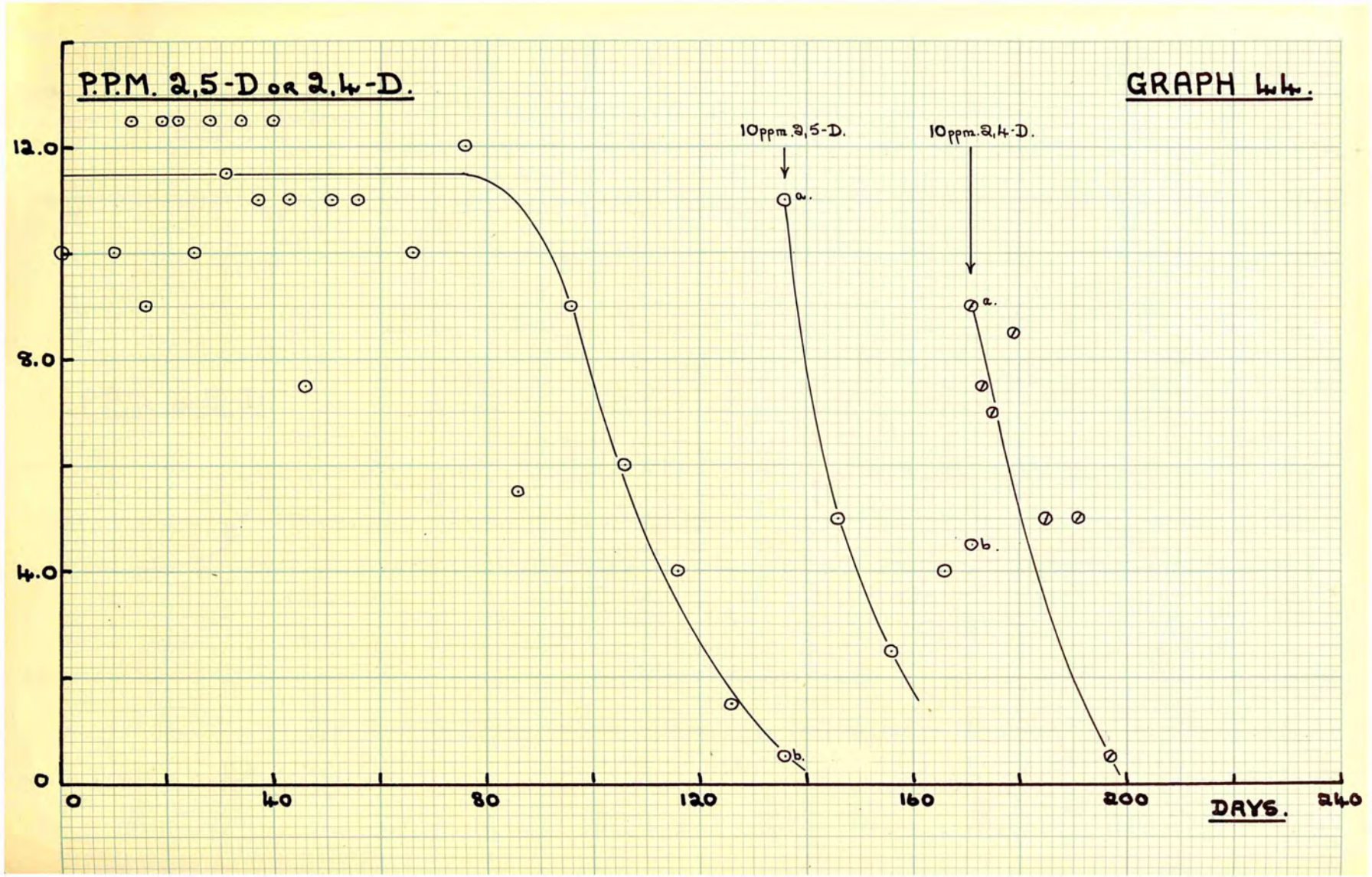
Two other perfusers (Graph 42a, Tables 42a, 42b,) were enriched to 100 ppm. 2,4-D and, when a rapid breakdown rate had been achieved, were switched to a mixture of 100 ppm. 2,4-D and 200 ppm. 2,4-DCP. The phenol went immediately into a slow breakdown phase. The rate of 2,4-D breakdown was slowed considerably but accelerated again, and approached completion, when a low phenol concentration had been reached. One of the perfusers (Graph 42a,) was refilled twice successively with 2,4-D and its rapid disappearance each time showed retention of high activity against this compound. Both perfusers were then refilled with the same 2,4-D/2,4-DCP mixture as before. In both cases 2,4-D and 2,4-DCP disappearance rates were higher though following the same sort of paths as before. A third refill with the mixture resulted in further acceleration of the 2,4-DCP breakdown rate and, to a lesser extent, that of the 2,4-D.

A series of three perfusers (Graph 42c, Tables 42c, 42d, 42e,) were all enriched to 100 ppm. 2,4-D then a mixture of 100 ppm.


P.P.M. 2,4-D OR 2,4-DCP.
GRAPH 4.2c.
100 ppm. 2,4-D.
100 ppm. 2,4-D + 100 ppm. 2,4-DCP.
Not drained, 12·5 ml. of 0·2% 2,4-DCP added.
Not drained, 15 ml. of 0·2% 2,4-DCP added.
100 ppm. 2,4-D.
100 ppm. 2,4-D + 100 ppm. 2,4-DCP,
Not drained, 12·5 ml. of 0.2% 2,4-DCP added.
150
100
50
0
0
20
40
60
80
100
120
DAYS.

2,4-D and 100 ppm. 2,4-DCP was added to each. The phenol disappeared rapidly from all three and, after replacing it without draining, again disappeared. Meanwhile, the 2,4-D had been going at a fairly high rate though somewhat slower than when alone in the perfuser. The same behaviour was observed on again refilling each perfuser with the same mixture. Subsequently, a succession of 2,4-D refills were decomposed rapidly showing retention of this adaptation. A succession of 2,4-DCP refills did likewise. It is of interest to note that at no point did a lag phase develop in the 2,4-DCP breakdown curve such as had characterised the 2,4-D followed by 2,4-DCP perfusers. This may have been due to the low concentration of both 2,4-D and 2,4-DCP used for inhibitory tendencies had been noted with high concentrations of both of these compounds in simple perfusions.


P.P.M. 2,5-D.
GRAPH 4.3.
120
100
80
60
0
20
40
60
80
100
120
DAYS.
P.P.M. 2,5-D.
GRAPH 4.3a.
180
140
100
60
20
0
20
40
60
80
100
120
DAYS.


P.P.M. 2,5-D or 2,4-D.
GRAPH 44.
10ppm. 2,5-D.
10ppm. 2,4-D.
a.
b.
12.0
8.0
4.0
0
0
40
80
120
160
200
240
DAYS.

2,5-dichlorophenoxyacetic acid (2,5-D).

This substance was reported as having a high physiological activity (125,). The Relative Toxicity as determined by cress assay was 8.5, a moderately high value.

Direct Perfusion.

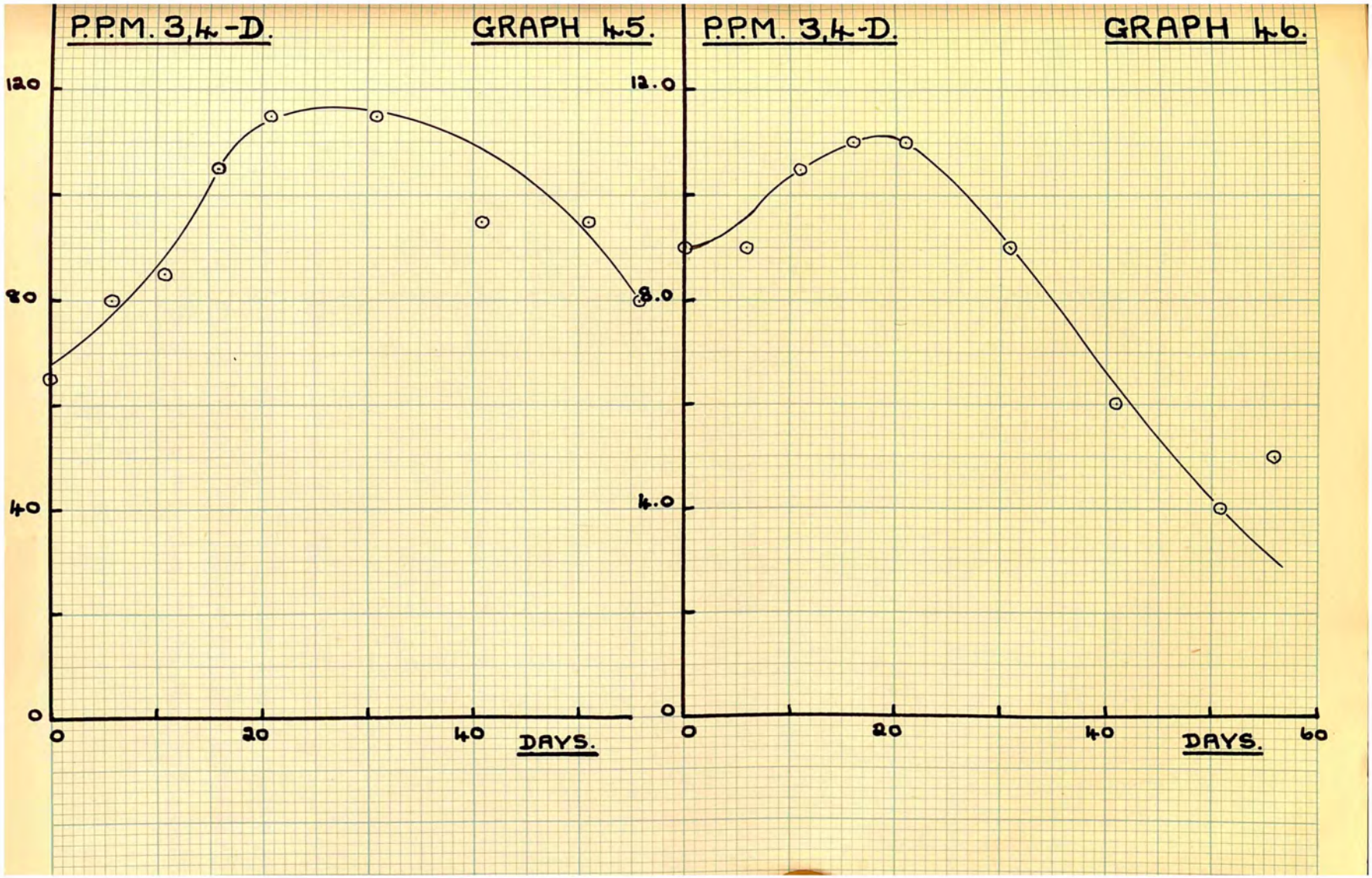
Perfusion at 100 ppm. produced none of the normal adaptation phenomena. In one perfuser (Graph 43,) there was no sign of definite adaptation in 90 days, only a slow loss of toxicity. With a second perfuser (Graph 43a,) there was an apparent 70% increase in toxicity during the first 25 days followed by a slow breakdown, to 40% of the initial activity, during the next 90 days. During this period the rate of disappearance was somewhat higher than the gradual loss of activity in the first perfuser. It is therefore possible that some degree of adaptation had occurred.

Two perfusers were operated at 10 ppm. 2,5-D and definite adaptation took place in about 40 to 70 days,(Graph 44, Tables 44, 44a,), followed by a slow but uniform breakdown. One perfuser (Graph 44,) was drained and again filled with 2,5-D at 10 ppm. Breakdown started immediately and at the same rate as before.

2,5-D followed by 2,4-dichlorophenoxyacetic acid (2,4-D).

The same perfuser (Graph 44,) adapted to 10 ppm. 2,5-D was again drained and then refilled with 10 ppm. 2,4-D. It was completely broken down in about 30 days.

Owing to the erratic nature of the assay results, it is not possible to say whether adaptation to the two compounds had been simultaneous or that a normal 2,4-D adaptation had subsequently taken place. In either case, the resultant rate of 2,4-D breakdown was exceedingly slow when compared with that for 10 ppm. 2,4-D in a directly enriched 2,4-D perfuser.

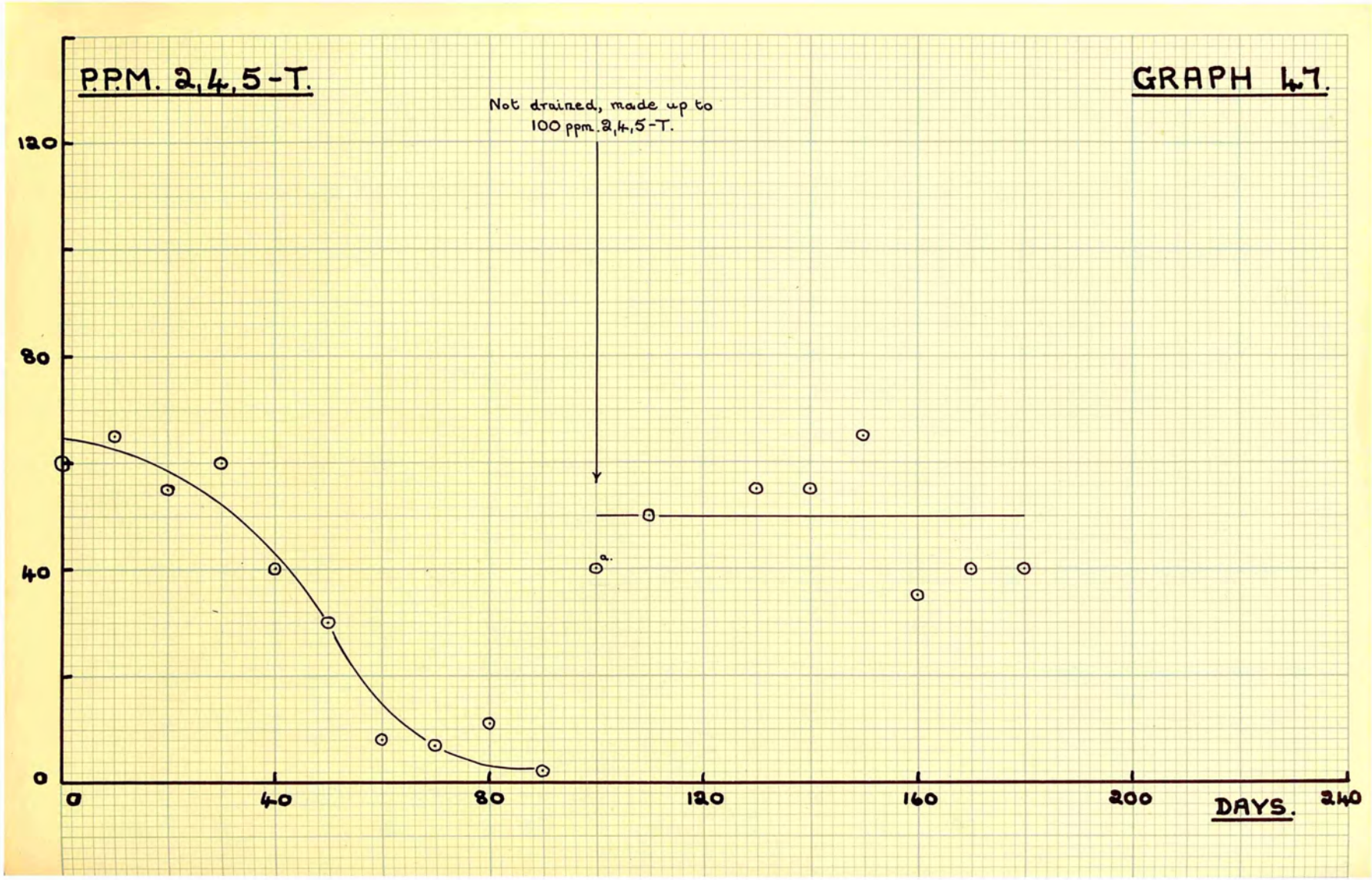

P.P.M. 3,4-D.
GRAPH 4.5.
120
80
40
0
0
20
40
DAYS.
P.P.M. 3,4-D.
GRAPH 4.6.
12.0
8.0
4.0
0
0
20
40
60
DAYS.

3,4-dichlorophenoxyacetic acid (3,4-D).

3,4-D has been said to have a fairly high (125,) or very high (63,) toxicity. As determined by the Cress Test, the Relative Toxicity had only the moderate value of 5.5.

Direct Perfusion.

Perfusion at both 10 and 100 ppm. resulted in adaptation after 20 to 30 days, preceded in each case by a rise in the apparent toxicity of the perfusate (Graphs 45, 46,). Here again, the most likely explanation is the production, and temporary accumulation, of a breakdown product more toxic to cress than the parent compound. The rate of breakdown at both concentrations was slow but was relatively faster in the 10 ppm. perfuser.


P.P.M. 2,4,5-T.
GRAPH 47.
Not drained, made up to
100 ppm. 2,4,5-T.
120
80
40
0
0
40
80
120
160
200
240
DAYS.

2,4,5-trichlorophenoxyacetic acid (2,4,5-T).

Very high physiological activity has been claimed for this compound (63, 85a, 95, 125,) and the fairly high Relative Toxicity of 17 is in agreement with these observations.

Direct Perfusion.

In two perfusions at 100 ppm. breakdown appeared to commence within 10 to 20 days and to cease after about 60 days when the original toxicity of the perfusate had been lowered to the equivalent of 5 to 10 ppm. 2,4,5-T. (Graph 47, Tables 47, 47a,). The perfusates were made up to 250 ml. using 100 ppm. 2,4,5-T solution and without draining. Activity was apparently lost for there was no significant breakdown in a subsequent 80 days.

4-chloro,2-methyl-phenoxyacetic acid (MCPA).

MCPA has been found to possess a very high physiological activity and Thomson et al (125,) found it to be much more toxic than 2,4-D. This result was also obtained with the cress assay technique which showed it to have a Relative Toxicity of approximately 140.

Direct Perfusion.

In only one perfuser was direct adaptation attempted (~~Graph and~~ Table 48,). After a lag of about 80 days, breakdown commenced fairly rapidly. With subsequent refills there was, at first, some tendency to further short lags before each breakdown. This was soon overcome and a fairly high breakdown rate achieved. Though the the perfusate concentration was nominally 100 ppm. each time, the assay results indicated starting concentrations of about half this value. It may be assumed that the stock solution had deteriorated with age. The initial lag of about 80 days compares well with the 70 days reported by Audus (9,).

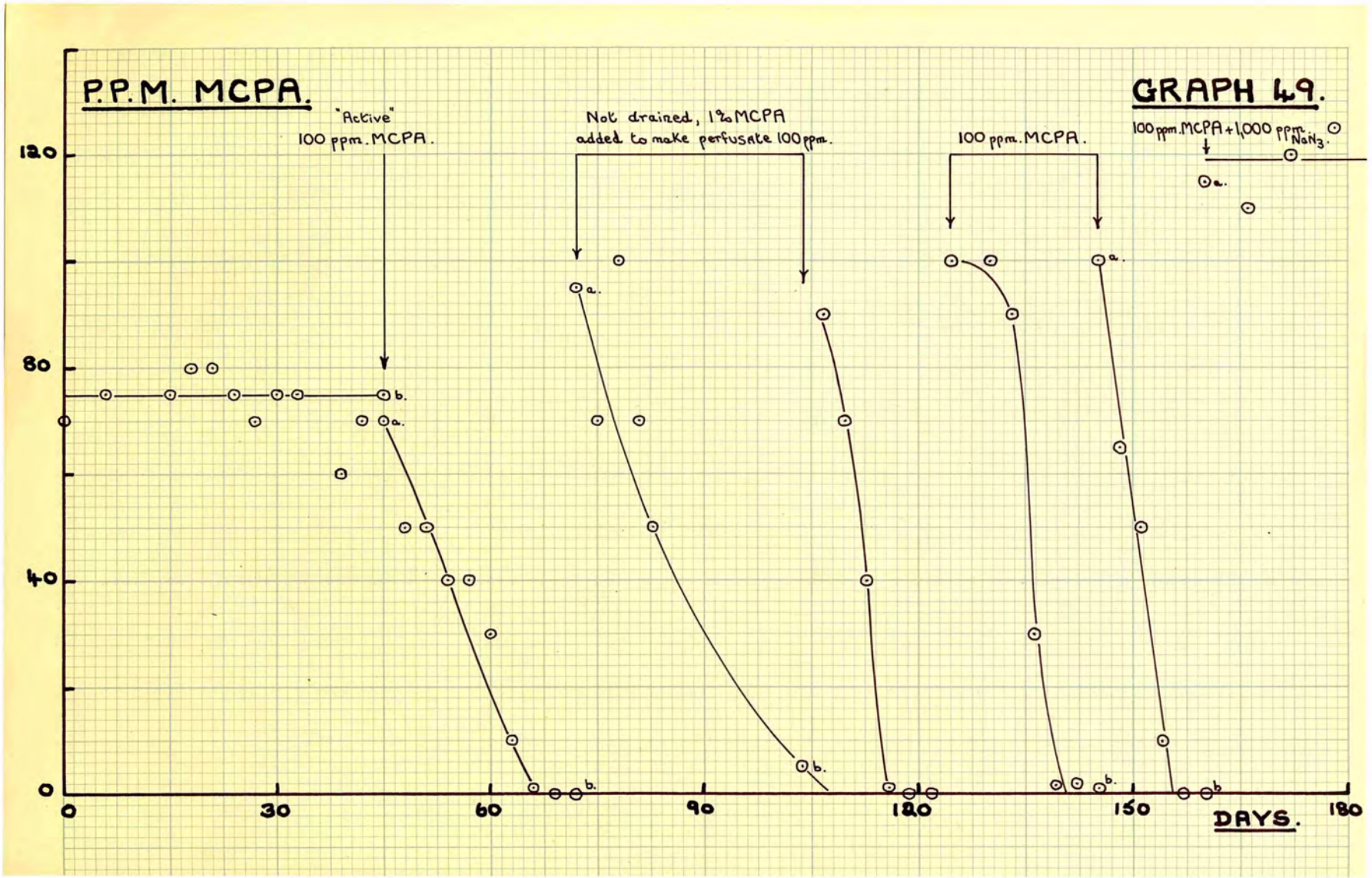
It is probable that the 2-methyl group of MCPA is fairly labile and subject to biological oxidation to give a carboxyl group. The partly oxidised molecule could then be considered to be a para-chloro derivative of phenoxyacetic acid or a meta-chloro derivative of benzoic acid, either of which might be expected to be physiologically active. If this oxidation product has a toxicity/concentration curve similar to that of unaltered MCPA, the apparently low starting concentrations might find an explanation. Moulds

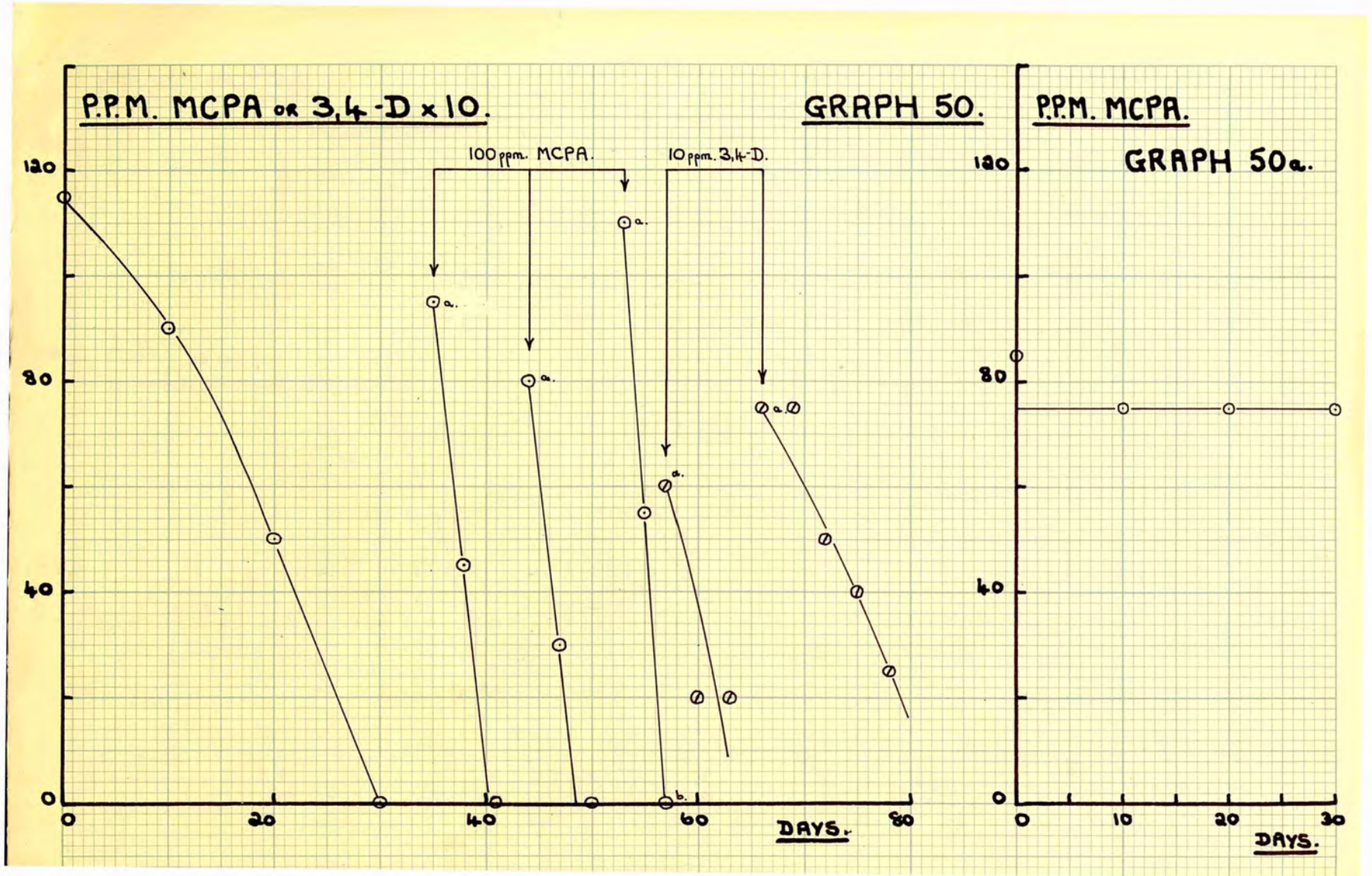
were often observed to grow for long periods in MCPA stock solution but only in small amounts and without marked effect on the concentration.

Assisted Enrichment (Transferred Adaptation).

Because of the long lag phase preceeding MCPA adaptation, direct adaptation was not resorted to for the production of enriched perfusers for cross- and simultaneous-adaptation experiments. All subsequent perfusers were produced directly, or indirectly, by the transferred adaptation technique from the only directly adapted perfuser and may be considered as sub-cultures of similar composition to that of the initial adapted population.

The behaviour of perfusers subjected to assisted enrichment conditions was fairly consistent. There was usually little evidence of a lag phase, breakdown commencing almost immediately and being complete in 20 to 30 days with 100 ppm. MCPA in the perfusate (Graphs 50, 51, 52, 53, 56, 57, 58, 59, 60, 61, Tables 50, 51, 51a, 51b, 52, 53, 56, 57, 57a, 57b, 57c, 57d, 58, 58a, 59, 59a, 60, 60a, 60b, 61, 61a, 61b,). In one exceptional case (Graph and Table 56,) the initial breakdown was complete in 5 days. The rate of breakdown accelerated during each initial stage and even further with subsequent refills till high and fairly constant rates were achieved. It was usually at this stage that the perfuser was drained and refilled with new substrate for cross-adaptation experiments.


P.P.M. MCPA.
GRAPH 49.
"Active"
100 ppm. MCPA.
Not drained, 1% MCPA
added to make perfusate 100 ppm.
100 ppm. MCPA.
100 ppm. MCPA + 1,000 ppm. NaN_3.
120
80
40
0
0
30
60
90
120
150
180
DAYS.
a.
b.

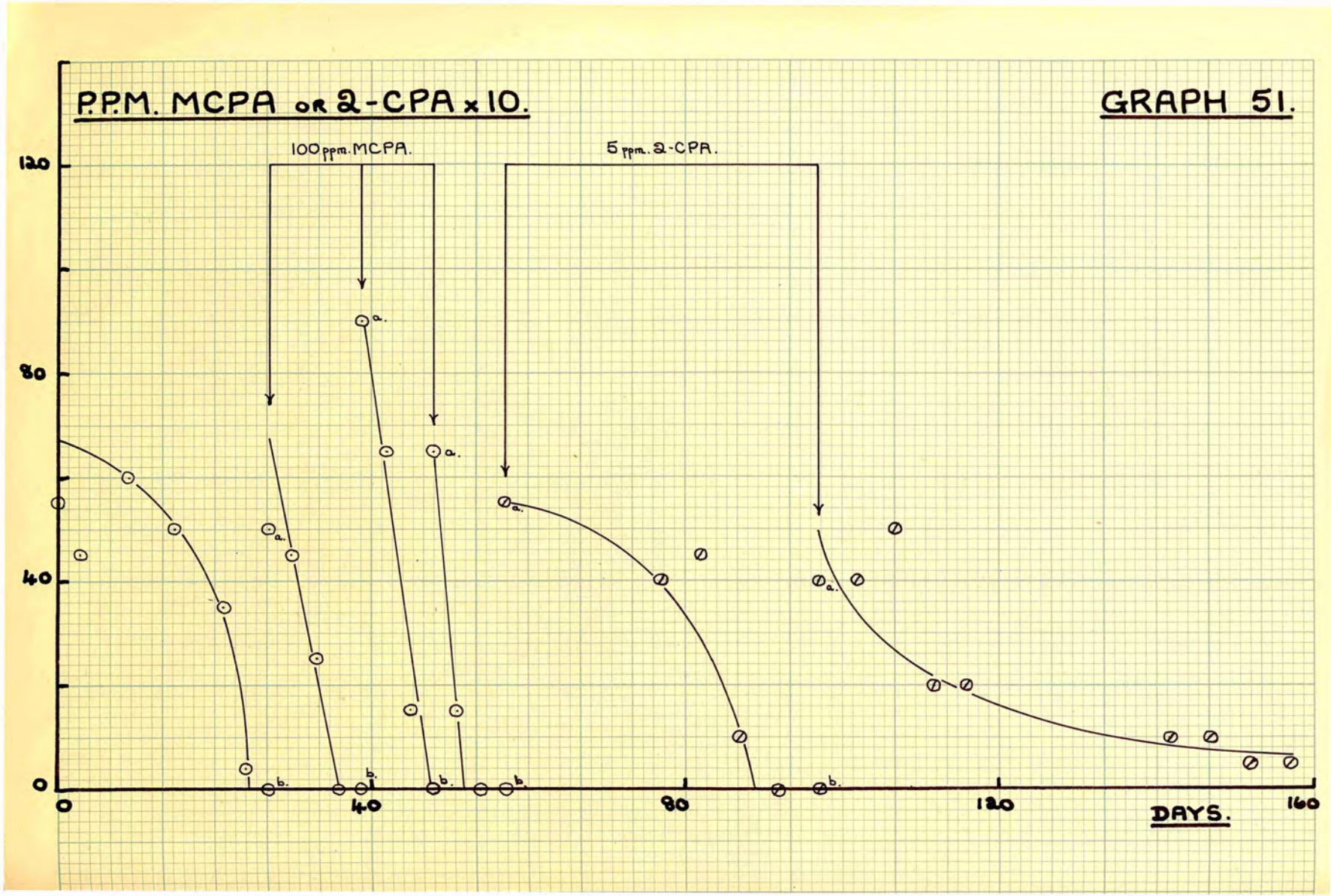

P.P.M. MCPA or 3,4-D x 10.
GRAPH 50.
100 ppm. MCPA.
10 ppm. 3,4-D.
120
80
40
0
0
20
40
60
80
DAYS.
a.
b.
P.P.M. MCPA.
GRAPH 50a.
120
80
40
0
0
10
20
30
DAYS.

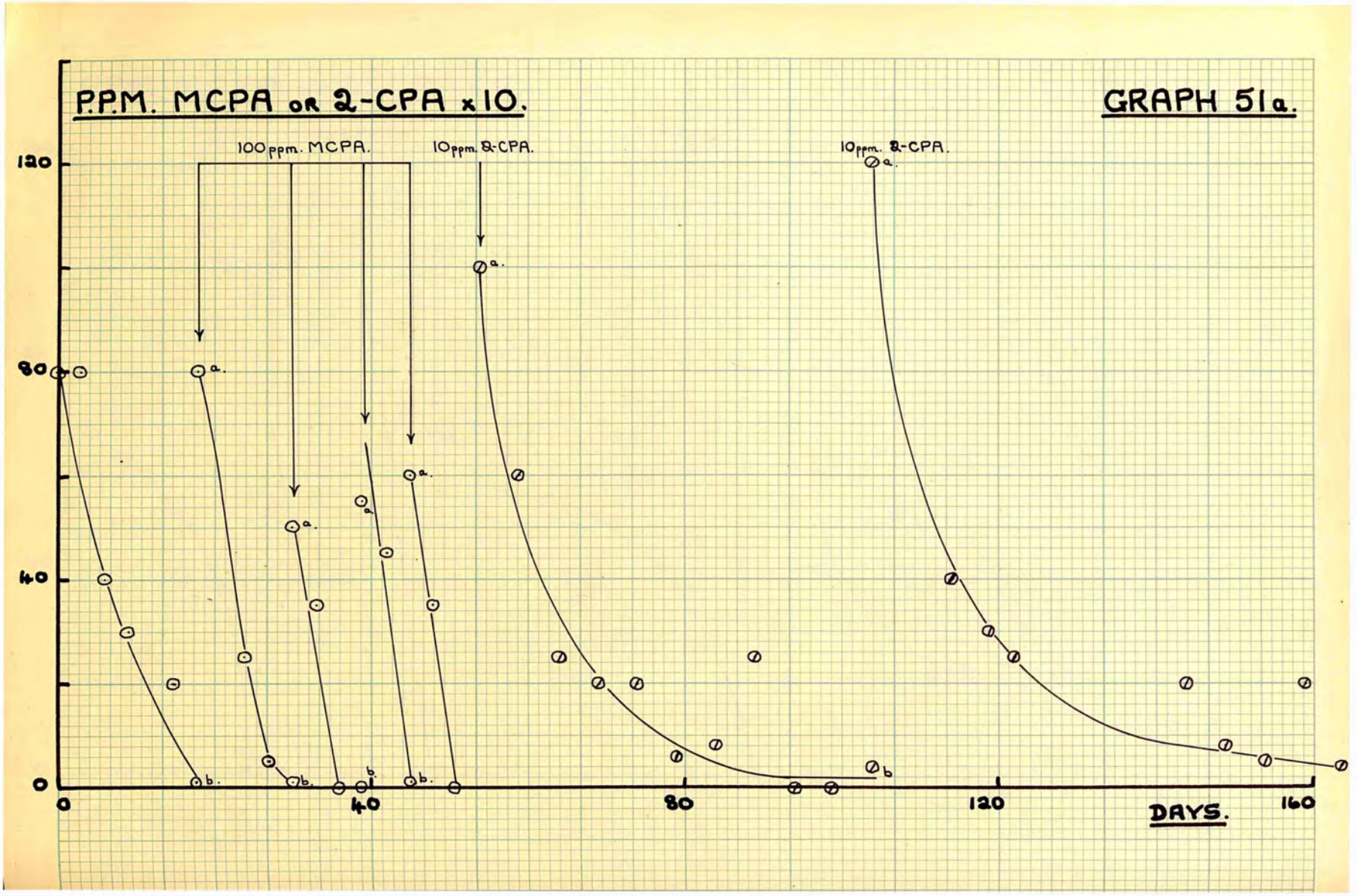
Accelerated enrichment of a perfuser containing crushed pot.

The first attempt to fix an MCPA adapted population onto sterilised, crushed pot failed (Graph and Table 49,). After 45 days the perfuser was drained and refilled with fresh 100 ppm. MCPA prepared from perfusate drained from another active perfuser. This time a population did become attached to the pot and breakdown was complete in about 20 days. In order not to lose adapted bacteria from the system, the next two rechargings were made by adding concentrated MCPA solution without draining. Breakdown was slow, but complete, each time. For the next two changes the perfuser was drained and refilled as usual. Even though some bacteria may, in this way, have been lost from the system the breakdown rates were slightly higher than before. This suggests possible inhibition of the bacteria by toxic breakdown products accumulating in the undrained perfuser. In each case the rate of breakdown was slow compared with an equivalent soil perfuser. Addition of 0.1% sodium azide resulted in complete inhibition of breakdown showing it to be a biological process.

The nature of the accelerated enrichment process.

The combined MCPA active perfusate drained from two perfusers was made up to 500 ml. of 100 ppm. MCPA with distilled water and MCPA stock solution. Half of this solution was put directly into a perfuser (Graph and Table 50,). The other was first allowed to simmer gently for 30 mins., cooled and added to another


P.P.M. MCPA OR 2-CPA x 10.
GRAPH 51.
100 ppm. MCPA.
5 ppm. 2-CPA.
120
80
40
0
a.
b.
0
40
80
120
160
DAYS.


P.P.M. MCPA OR 2-CPA x 10.
GRAPH 51a.
100 ppm. MCPA.
10 ppm. 2-CPA.
10 ppm. 2-CPA.
120
80
40
0
a.
b.
0
40
80
120
160
DAYS.

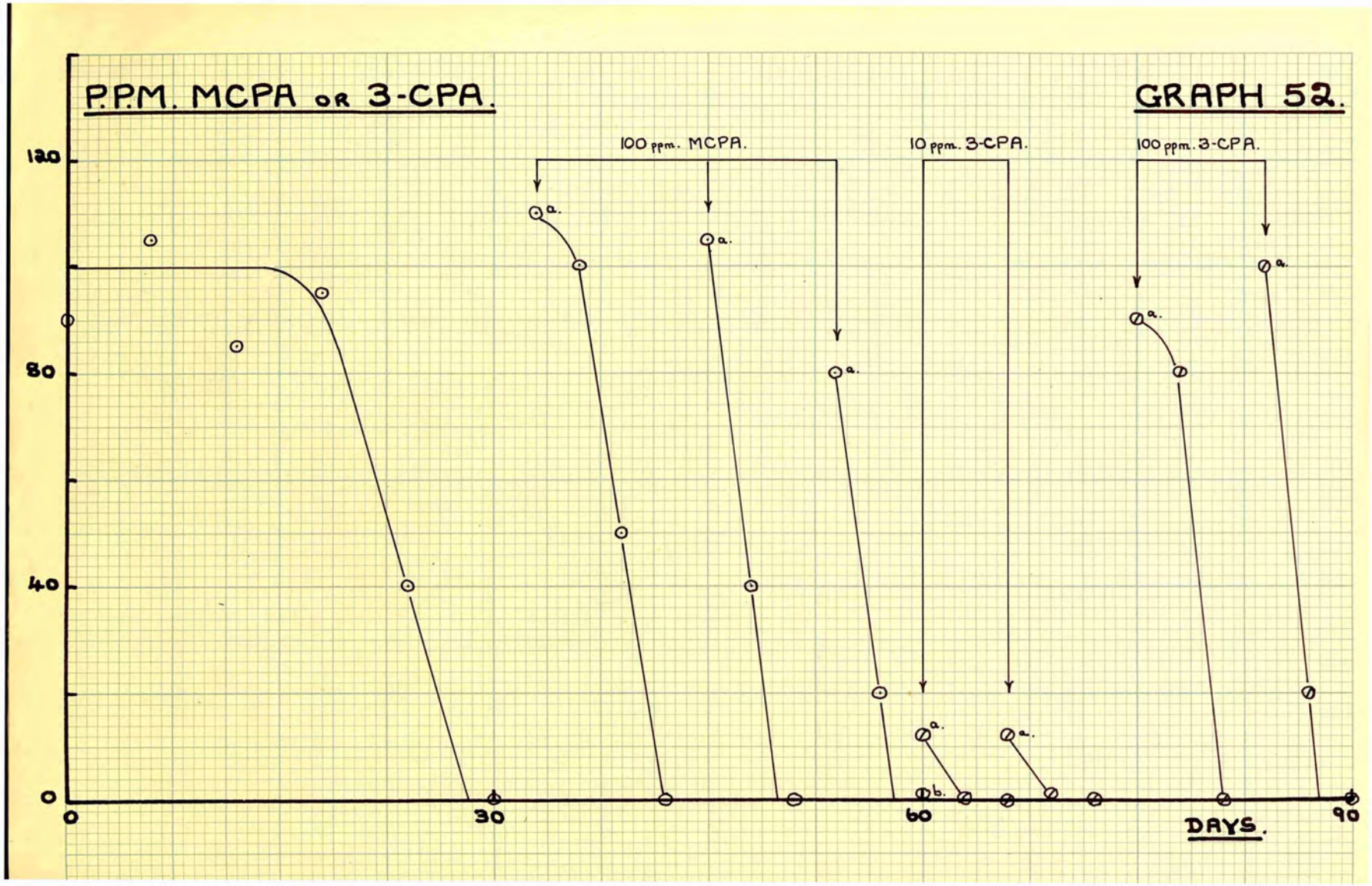
perfuser (Graph and Table 50a,). Breakdown of the MCPA commenced immediately in the first perfuser and was completed in 30 days while the MCPA concentration in the second perfuser remained unchanged during this period. From the results of this experiment it may be concluded that accelerated enrichment is brought about by the transfer of adapted organisms which quickly develop into a new active population or that some thermolabile breakdown product or metabolite in the perfusate can stimulate the rapid adaptation and multiplication of normal cells in the fresh soil. The former is the more likely explanation and is supported by the result of the pot enrichment experiment (Graph and Table 49,).

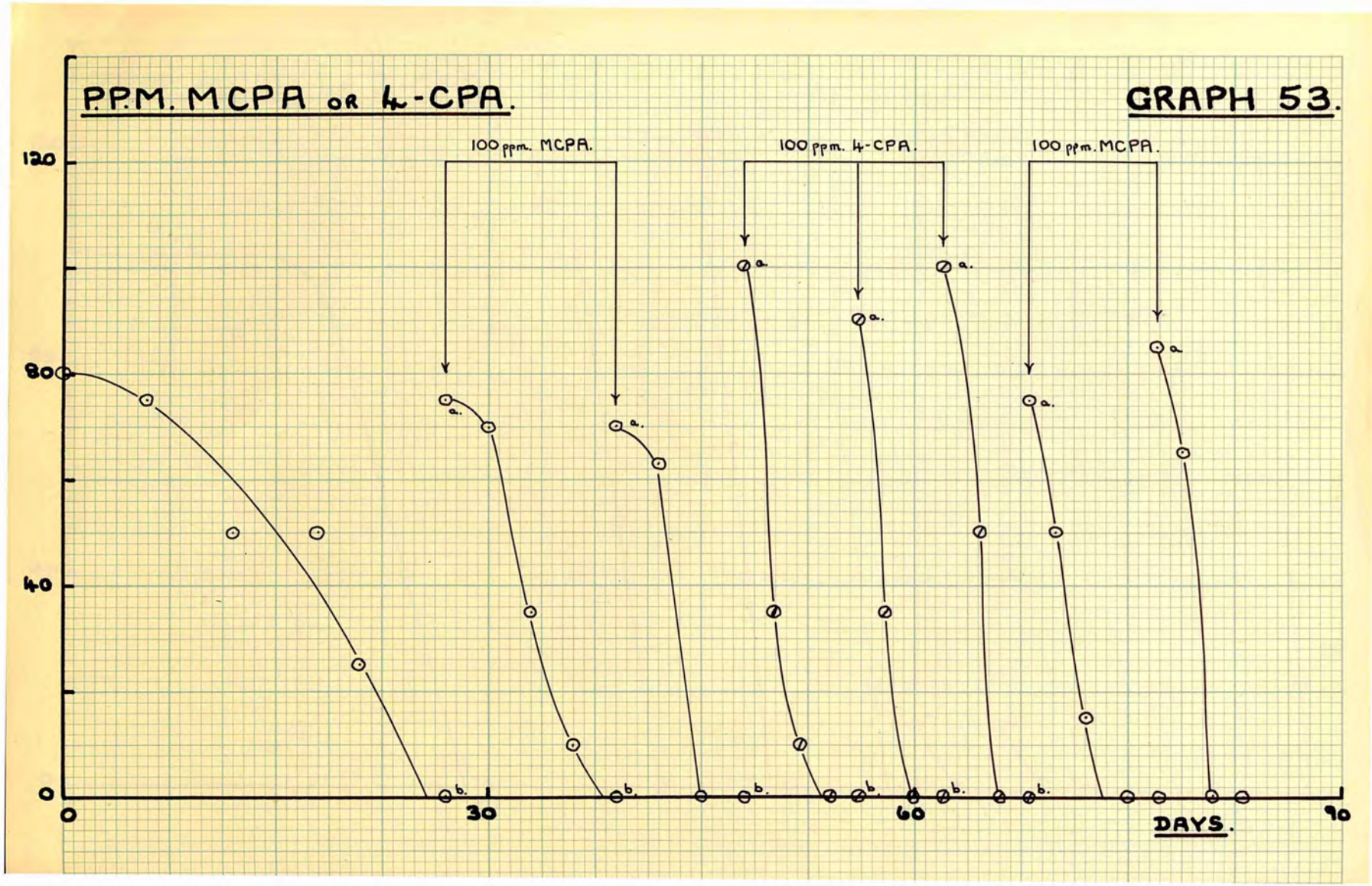
MCPA followed by 2-chlorophenoxyacetic acid (2-CPA).

When 5 ppm. 2-CPA was added to a perfuser enriched to 100 ppm. MCPA (Graph and Table 51,) there was some evidence of slow breakdown over a period of 20 days followed by a rapid breakdown to zero concentration. On refilling at 5 ppm. breakdown continued at the initial slow rate.

10 ppm. 2-CPA added to a 100 ppm. MCPA enriched perfuser produced a somewhat different behaviour pattern in which the rapid disappearance phase came first, followed by slow breakdown to completion. On refilling at 10 ppm. the same behaviour was observed (Graph and Table 51a,).

A third MCPA enriched perfuser (Table 51b,) was first refilled with 10 ppm. 2-CPA and behaved as in Graph 51a. On draining and refilling at 5 ppm., breakdown followed the same course as


P.P.M. MCPA OR 3-CPA.
GRAPH 52.
100 ppm. MCPA.
10 ppm. 3-CPA.
100 ppm. 3-CPA.
120
80
40
0
0
30
60
90
DAYS.
a.
b.


P.P.M. MCPA OR 4-CPA.
GRAPH 53.
100 ppm. MCPA.
100 ppm. 4-CPA.
100 ppm. MCPA.
120
80
40
0
0
30
60
90
DAYS.
a.
b.

in Graph 51. A third refill at an intermediate concentration showed little real evidence of breakdown.

MCPA followed by 3-chlorophenoxyacetic acid (3-CPA).

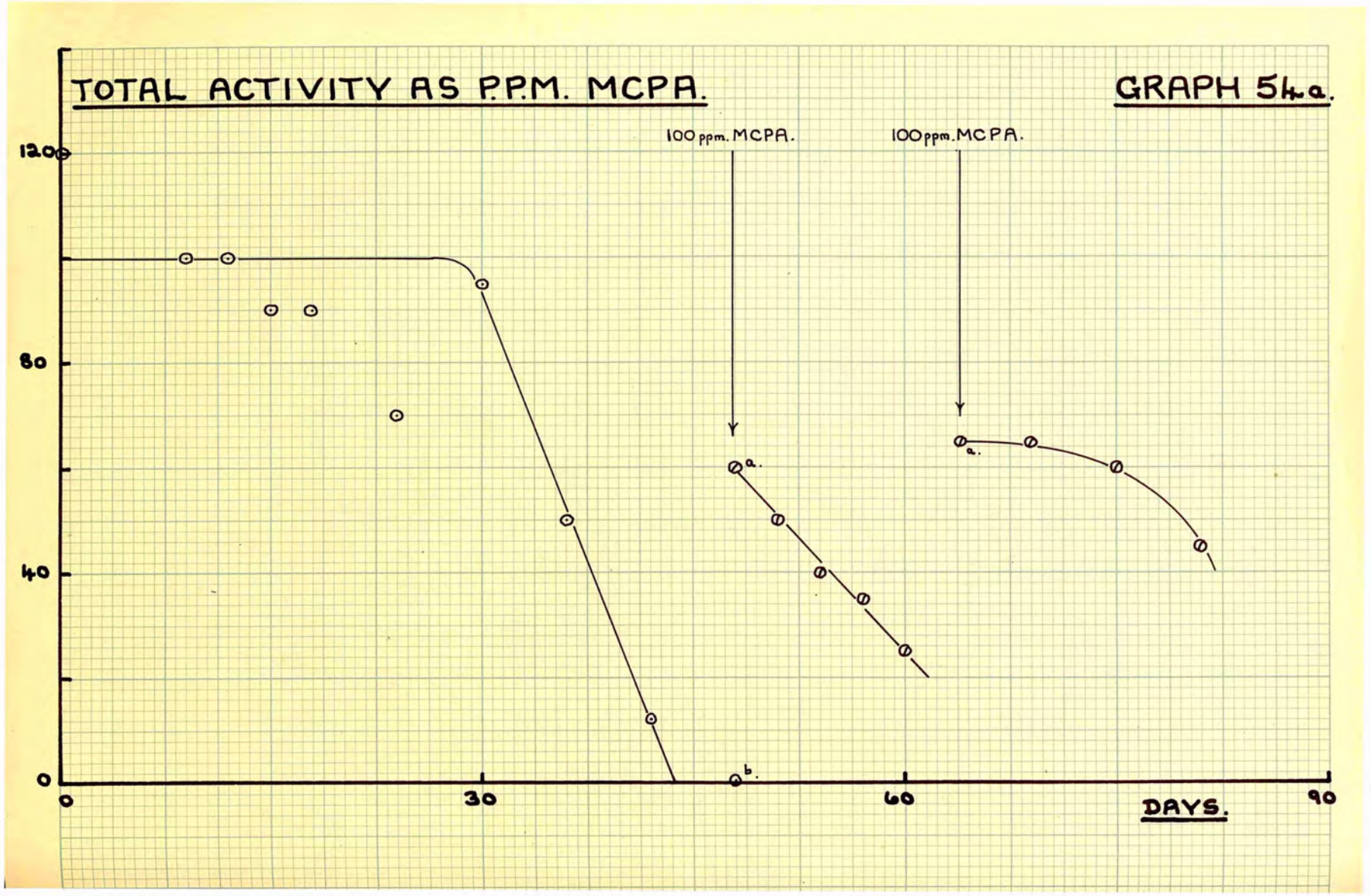
Rapid breakdown of 3-CPA at 10 ppm. occurred when it was added to a 100 ppm. MCPA enriched perfuser and went on to completion (Graph and Table 52,). After another charge at 10 ppm. two refills at 100 ppm. were made and these were likewise broken down rapidly and completely.

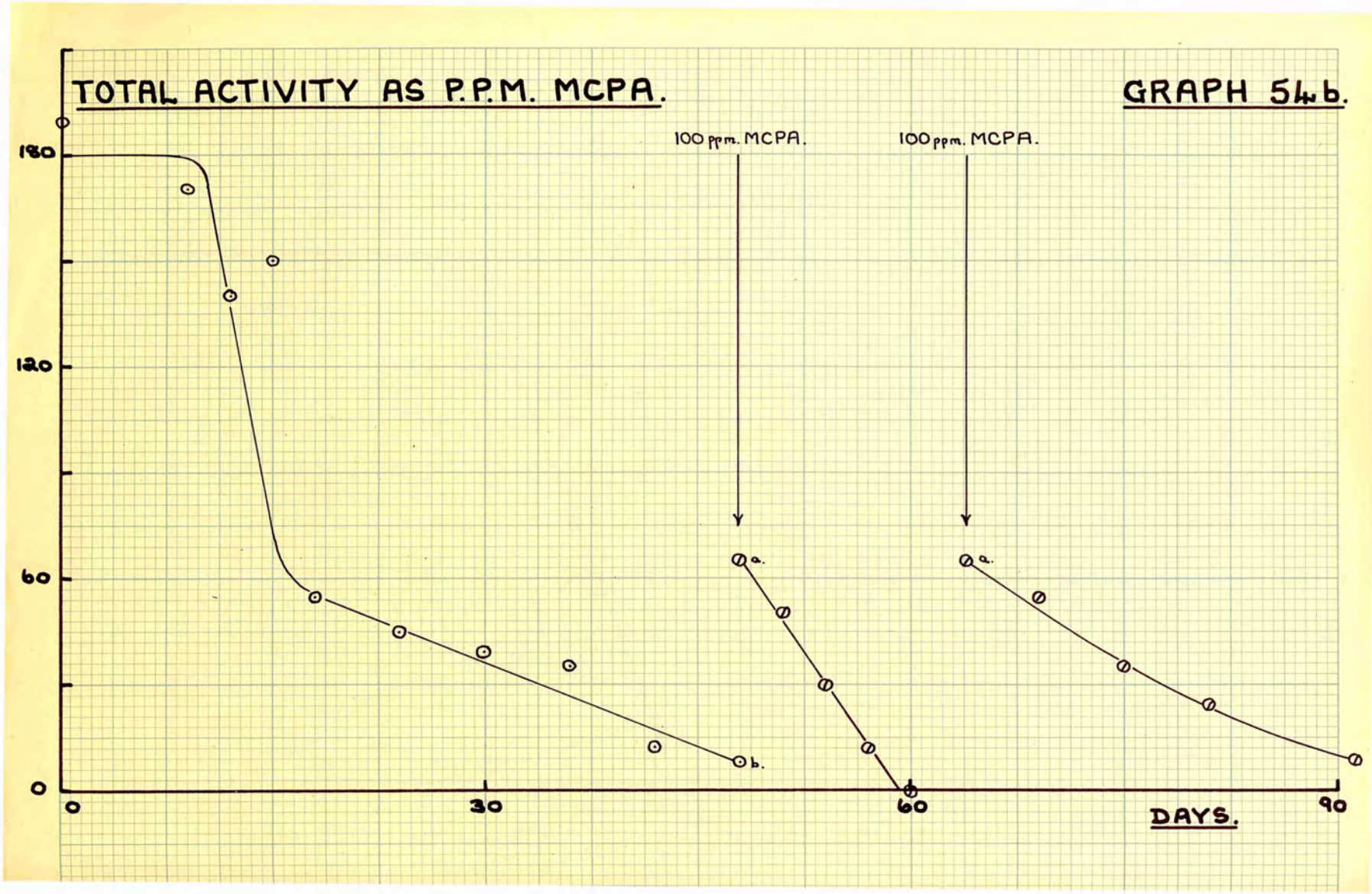
MCPA followed by 4-chlorophenoxyacetic acid (4-CPA).

4-CPA at 100 ppm. added to a 100 ppm. MCPA enriched perfuser disappeared rapidly, completely and without lag (Graph and Table 53,). With subsequent refills at the same concentration the very high rate was maintained. That adaptation to MCPA had been retained during this time was shown by the rapid breakdown on draining and refilling with this compound at 100 ppm.

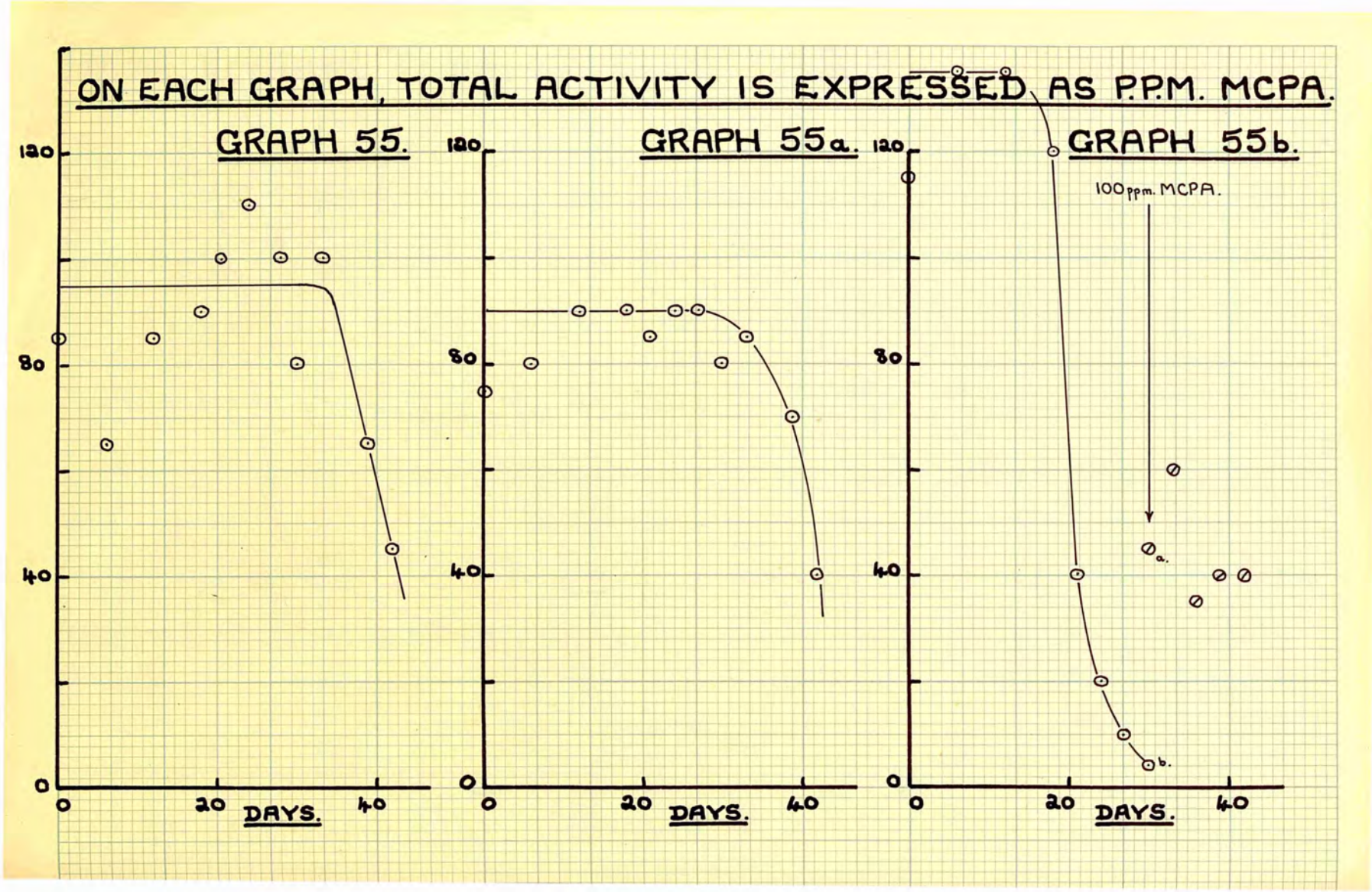
Attempts to shorten MCPA lag by direct perfusion in mixture with 4-CPA and 2,4-D.

From a common stock solution of 100 ppm. MCPA, three solutions were prepared, each to contain 100 ppm. MCPA along with 1, 10, or 100 ppm. 4-CPA respectively. These solutions were perfused through three similar perfusers


TOTAL ACTIVITY AS P.P.M. MCPA.
GRAPH 54a.
100 ppm. MCPA.
100 ppm. MCPA.
120
80
40
0
0
30
60
90
DAYS.
a.
a.
b.


TOTAL ACTIVITY AS P.P.M. MCPA.
GRAPH 54b.
100 ppm. MCPA.
100 ppm. MCPA.
180
120
60
0
a.
a.
b.
0
30
60
90
DAYS.

ON EACH GRAPH, TOTAL ACTIVITY IS EXPRESSED AS P.P.M. MCPA.



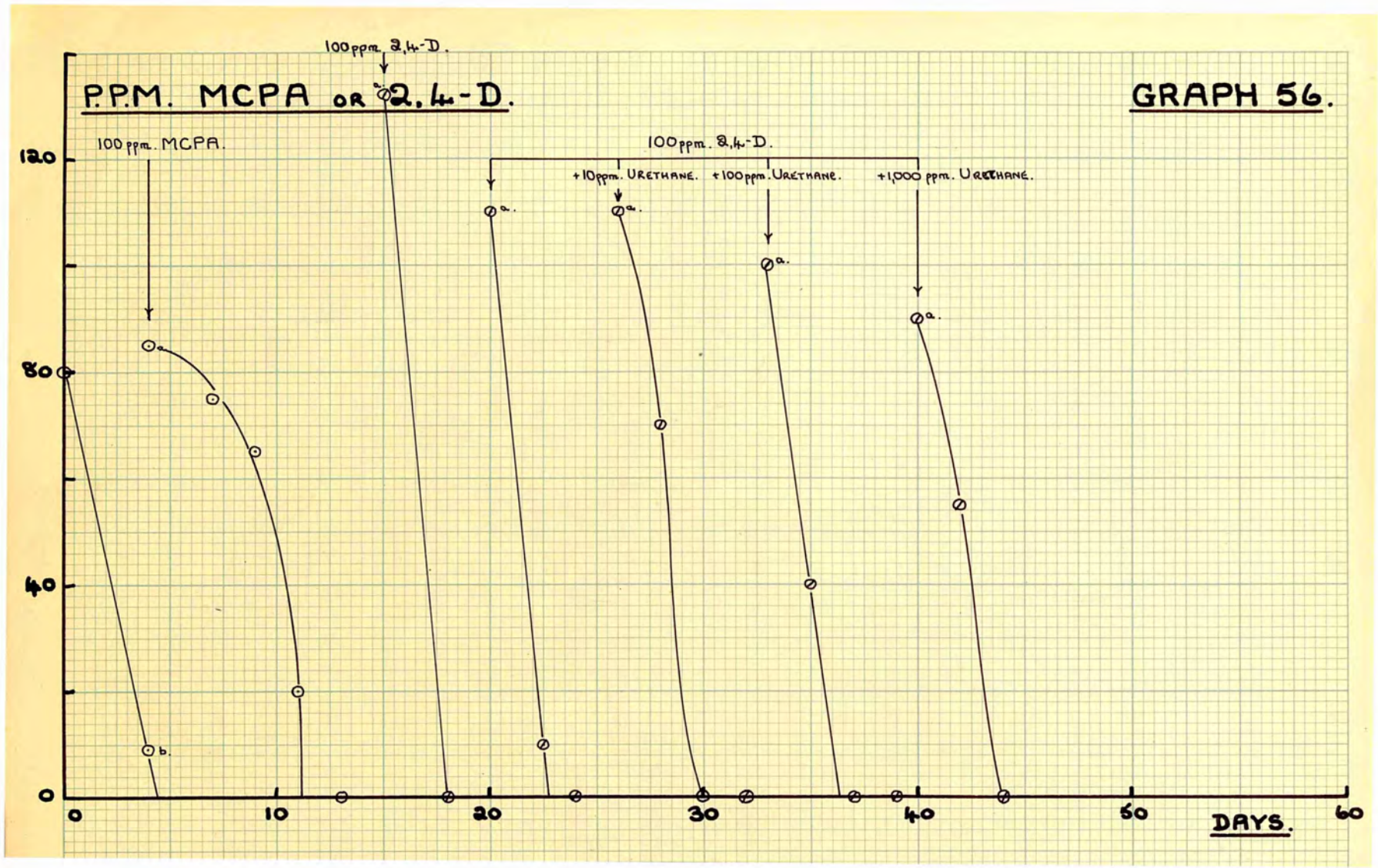
containing soil from a common batch. With the 1 and 10 ppm. 4-CPA mixtures a lag phase lasting some 20 days was followed by an accelerating rate of breakdown (Graph 54a, Tables 54, 54a,) followed to completion in the 10 ppm. perfuser. On refilling this perfuser with 100 ppm. MCPA, breakdown followed a linear but slower path. A further refill resulted in some loss of activity but breakdown recommenced after a lag of about 10 days.

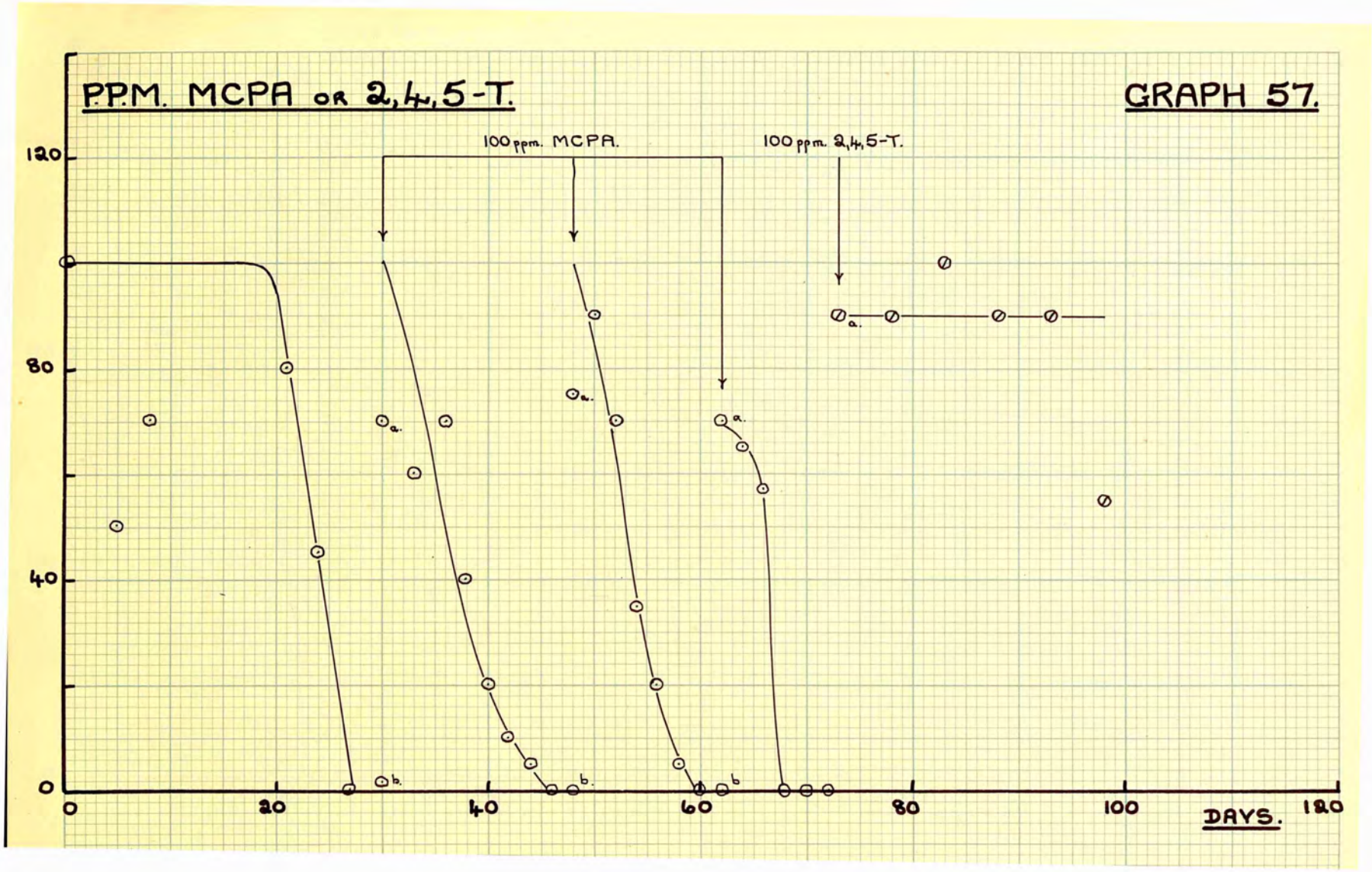
With the perfuser containing 100 ppm. each of 4-CPA and MCPA (Graph and Table 54b,) there was an initial lag of about 8 days (the normal for 4-CPA) followed by a rapid fall in perfusate toxicity to a level lower than that required by 100 ppm. MCPA. The rate then decelerated rapidly and continued linearly till all toxicity had gone, in about 50 days. A refill of 100 ppm. MCPA disappeared at a moderately rapid rate and a further one at a somewhat slower rate.

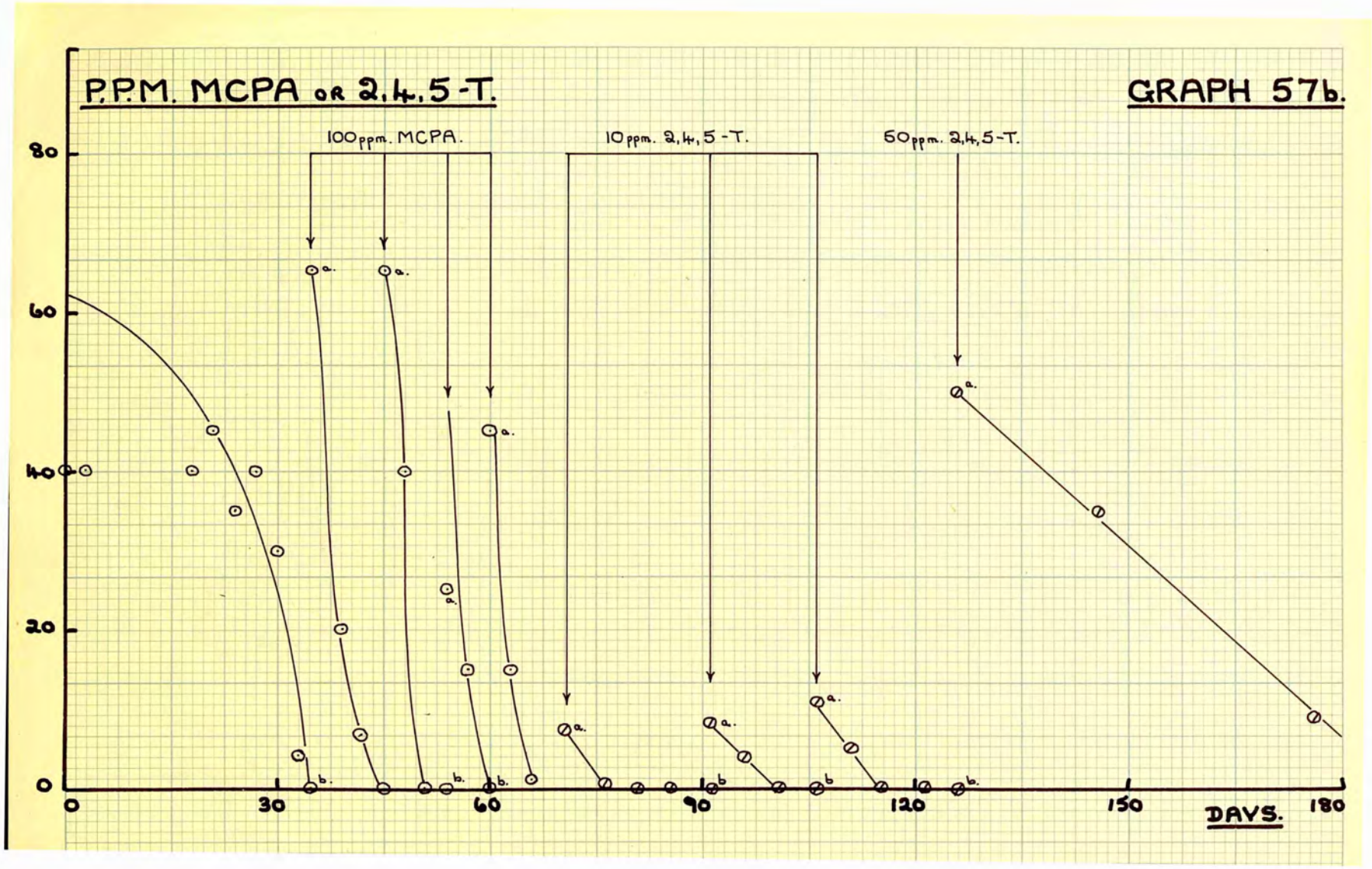
A mixture of 100 ppm. MCPA with 10 ppm. each of 4-CPA and 2,4-D (Graph and Table 55,) showed no sign of breakdown till 35 days had elapsed. A rapid reaction then set in but was not followed to completion. This lag was longer than those of 4-CPA and 2,4-D but much shorter than that of MCPA.

Omitting 4-CPA from the above mixture (leaving 100 ppm. MCPA and 10 ppm. 2,4-D) did not alter the behaviour for the lag of 35 days and subsequent rapid breakdown was again observed (Graph and Table 55a,).

A perfuser containing 100 ppm. each of MCPA and 2,4-D (Graph and Table 55b,) showed a lag of about 15 days (normal for


100ppm. 2,4-D.
P.P.M. MCPA OR 2,4-D.
GRAPH 56.
100 ppm. MCPA.
100ppm. 2,4-D.
+10ppm. URETHANE.
+100ppm. URETHANE.
+1,000 ppm. URETHANE.
a.
b.
120
80
40
0
0
10
20
30
40
50
60
DAYS.


P.P.M. MCPA OR 2,4,5-T.
GRAPH 57.
100 ppm. MCPA.
100 ppm. 2,4,5-T.
120
80
40
0
0
20
40
60
80
100
120
DAYS.
a.
b.


P.P.M. MCPA OR 2,4,5-T.
GRAPH 57b.
100ppm. MCPA.
10ppm. 2,4,5-T.
50ppm. 2,4,5-T.
80
60
40
20
0
0
30
60
90
120
150
180
DAYS.
a.
b.

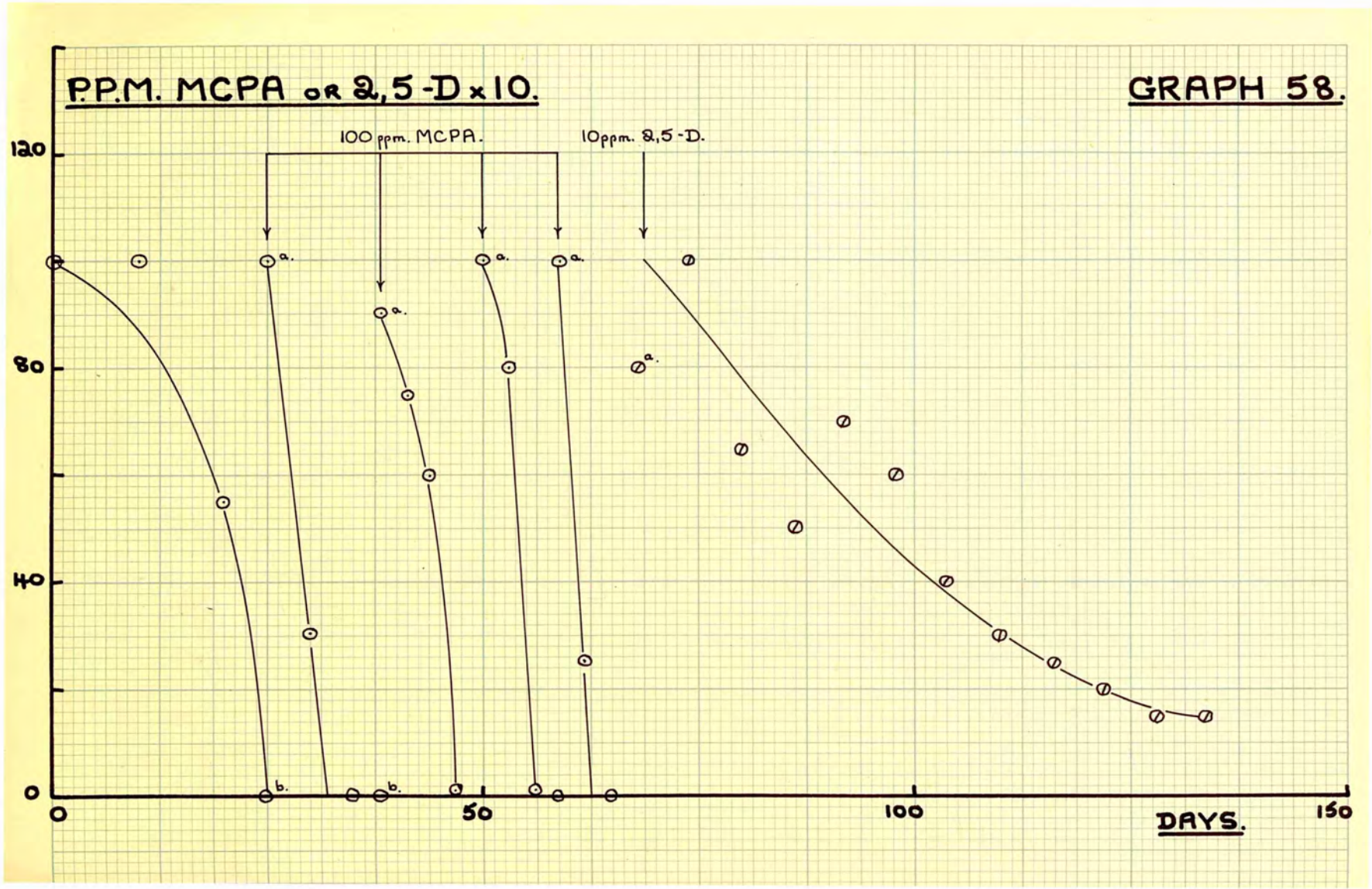
2,4-D) followed by rapid breakdown which decelerated after about 22 days when about 40% of the initial toxicity remained. Complete breakdown was achieved by the 30 th. day. After refilling with approximately 50 ppm. MCPA there was no evidence of breakdown in the next 12 days.
Adaptation to both 4-CPA and 2,4-D seems to be prolonged by the presence of MCPA while the lag of this compound is somewhat shortened.

MCPA followed by 2,4-dichlorophenoxyacetic acid (2,4-D).

When 100 ppm. 2,4-D was added to a perfuser enriched to 100 ppm. MCPA it disappeared quickly with no detectable lag (Graph and Table 56,). Further refills of 100 ppm. 2,4-D disappeared equally rapidly and neither 10, 100 nor 1,000 ppm. urethane had any measureable effect on the breakdown.

MCPA followed by 2,4,5-trichlorophenoxyacetic acid (2,4,5-T).

At 100 ppm., 2,4,5-T showed no sign of disappearance in 25 days when added to a perfuser enriched to 100 ppm. MCPA (Graph and Table 57,). At 10 ppm., 2,4,5-T always broke down at moderate speed, without lag, when added to such a perfuser (Graph 57b, Tables 57a, 57b, 57c, 57d,). The rate of breakdown always decreased as the toxicity approached zero. The ability to break down 2,4,5-T was retained through several changes of perfusate at 10 ppm. (Graph 57b, Tables 57a, 57b, 57c,). It was even possible to step up the perfusate concentration as far as 50 and 100 ppm.


P.P.M. MCPA OR 2,5-D x 10.
GRAPH 58.
100 ppm. MCPA.
10 ppm. 2,5-D.
120
80
40
0
0
50
100
150
DAYS.
a.
b.

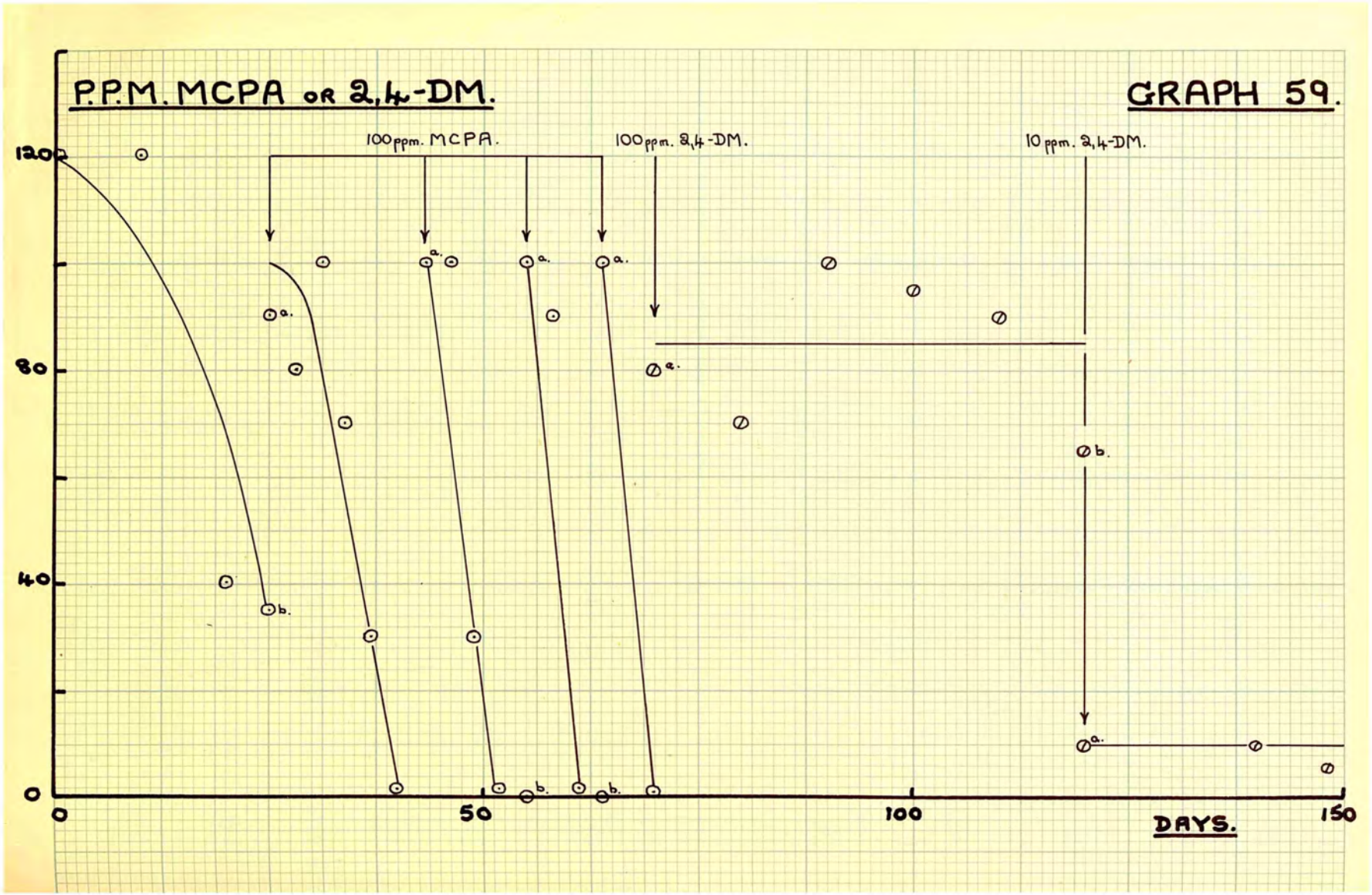
without inhibiting the 2,4,5-T breakdown.

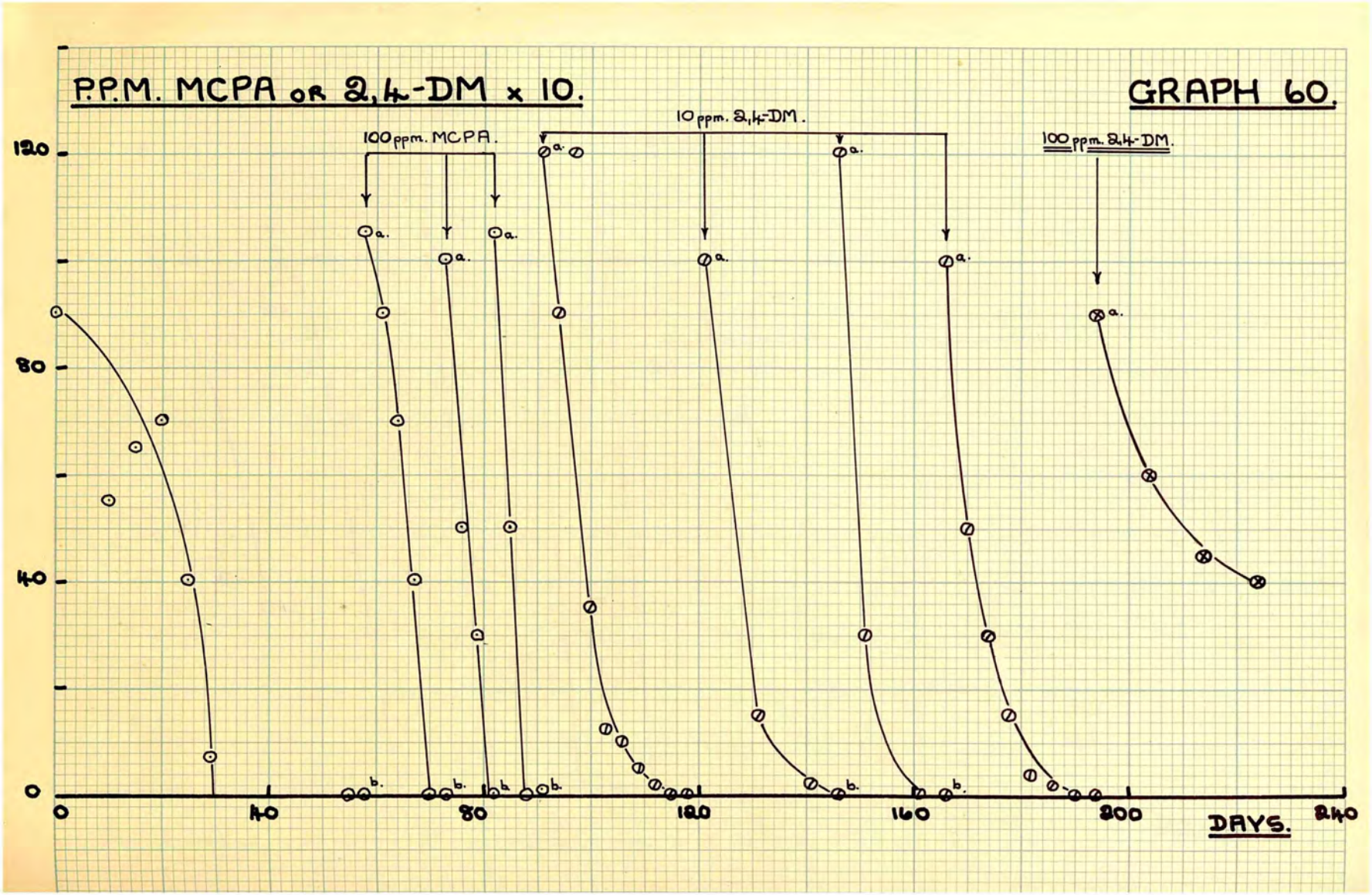
MCPA followed by 2,5-dichlorophenoxyacetic acid (2,5-D).

Two perfusers enriched to 100 ppm. MCPA were drained and each refilled with 10 ppm. 2,5-D. Qualitatively the same result was produced in each case. An initial rapid breakdown rate gradually decelerated as the toxicity of the perfusate approached zero, which was reached in 50 and 80 days respectively (Graph 58, Tables 58, 58a,).
The intermediate breakdown products of 2,5-D and 2,4,5-T are probably very similar and if toxic to the breakdown bacteria could cause the decelerating breakdown rate with each compound. The 2,5-dichloro combination seems to be the responsible factor for 4-chloro substituted compounds were, on the whole fairly labile and less prone to toxic effects. Another possible explanation of the decelerating breakdown is that the MCPA adapted enzyme system does not have a high affinity for these other substrates. The degree of absorbtion and rate of breakdown will depend on the substrate concentration. As the concentration falls, so will the rate of breakdown.

MCPA followed by 3,4-dichlorophenoxyacetic acid.(3,4-D).

10 ppm. 3,4-D was rapidly broken down, without lag, when added to a perfuser enriched to 100 ppm. MCPA. A second charge at 10 ppm. was also broken down though at a slightly reduced rate (Graph and Table 50,).

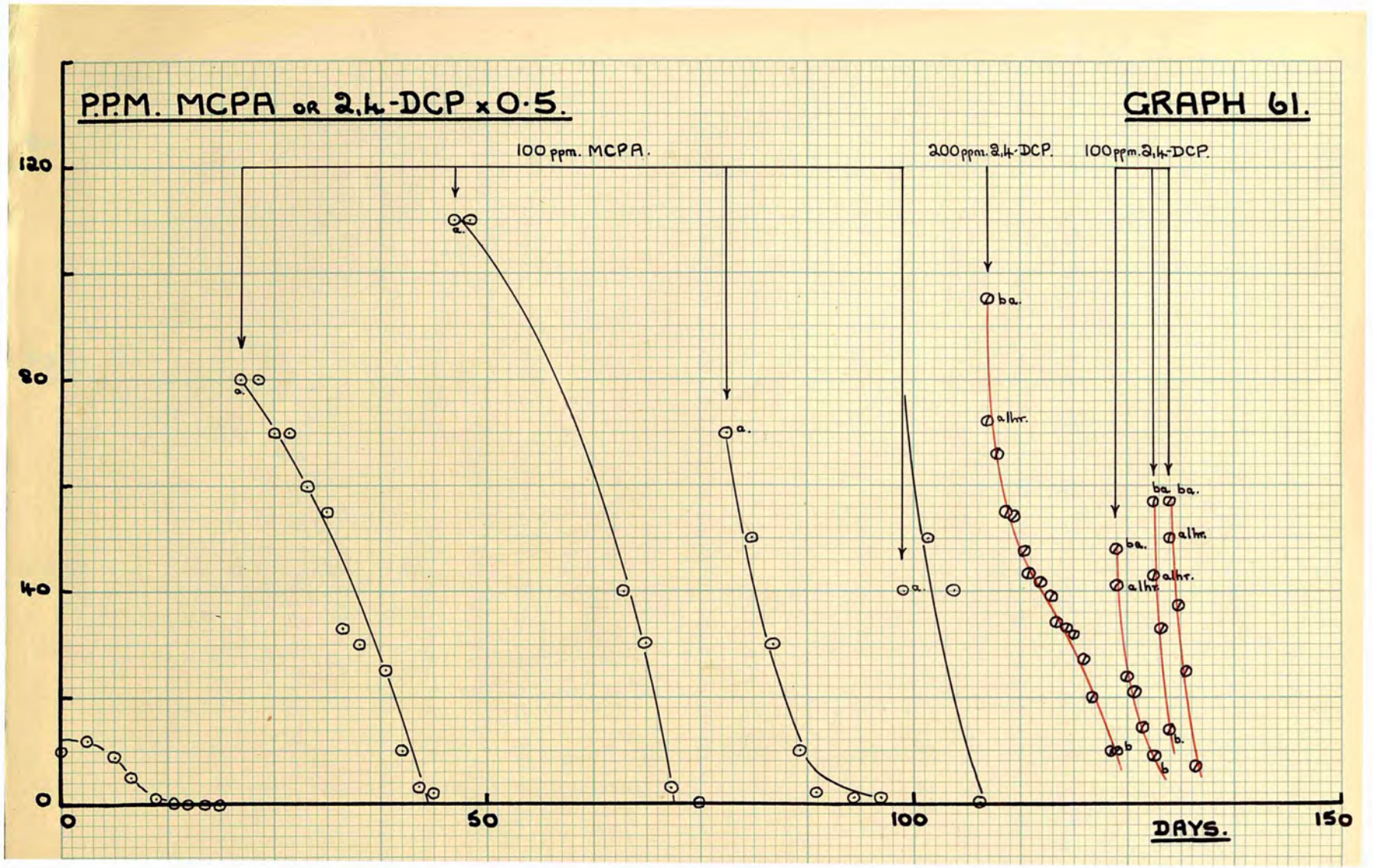

P.P.M. MCPA OR 2,4-DM.
GRAPH 59.
100 ppm. MCPA.
100 ppm. 2,4-DM.
10 ppm. 2,4-DM.
120
80
40
0
0
50
100
150
DAYS.
a.
b.


P.P.M. MCPA OR 2,4-DM x 10.
GRAPH 60.
100 ppm. MCPA.
10 ppm. 2,4-DM.
100 ppm. 2,4-DM.
120
80
40
0
a.
b.
0
40
80
120
160
200
240
DAYS.

MCPA followed by 2,4-dimethylphenoxyacetic acid (2,4-DM).

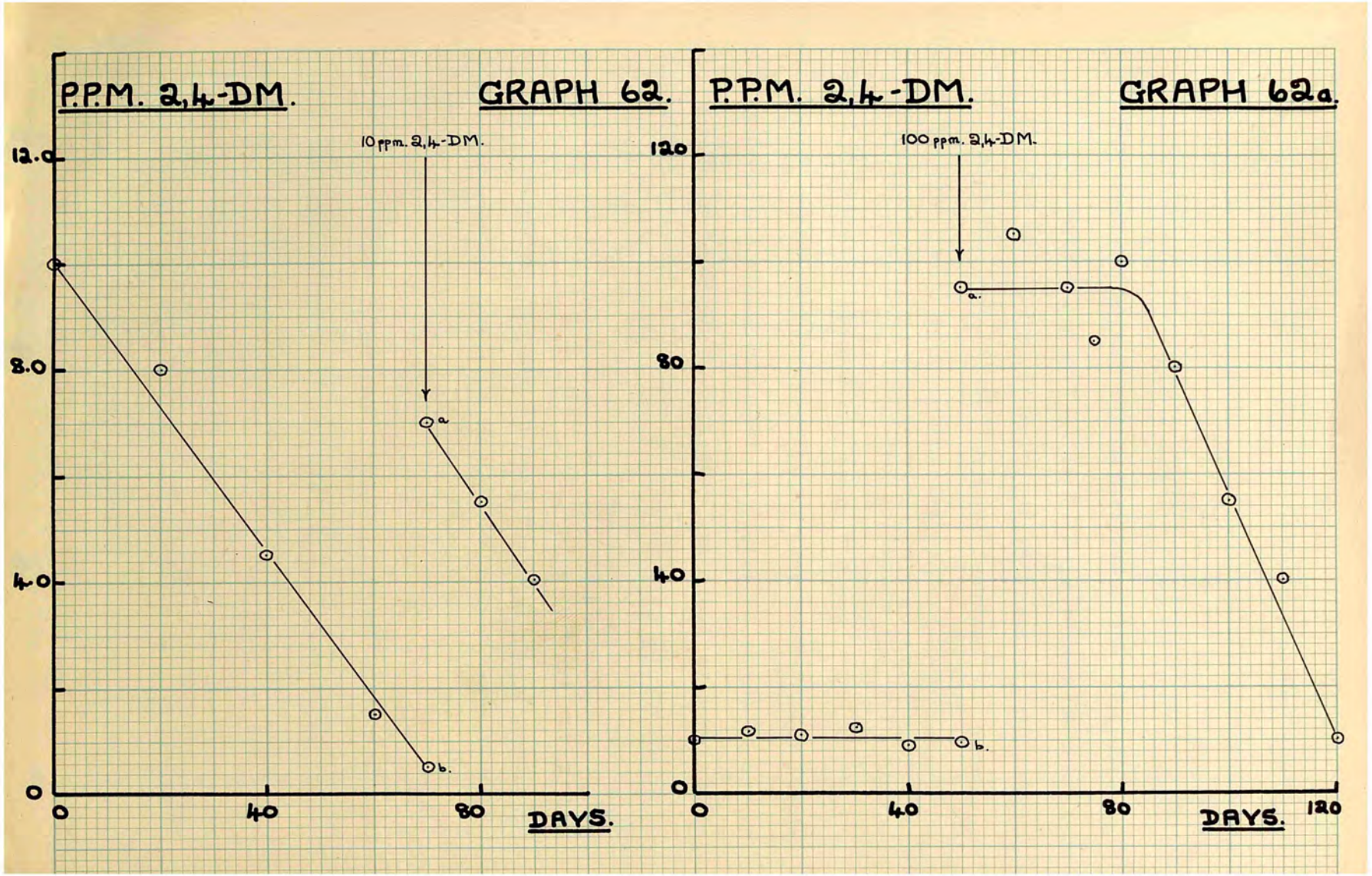
There was no evidence of breakdown of 100 ppm. 2,4-DM when added to a 100 ppm. MCPA enriched perfuser. When these two perfusers (Graph 59, Tables 59,59a,) were switched to 10 ppm. 2,4-DM, one (Graph and Table 59,) showed no sign of breakdown in a further 30 days but the other (Table 59a,) showed complete breakdown in 60 days. There was possibly an intermediate rise in perfusate toxicity reaching a peak after about 25 days.

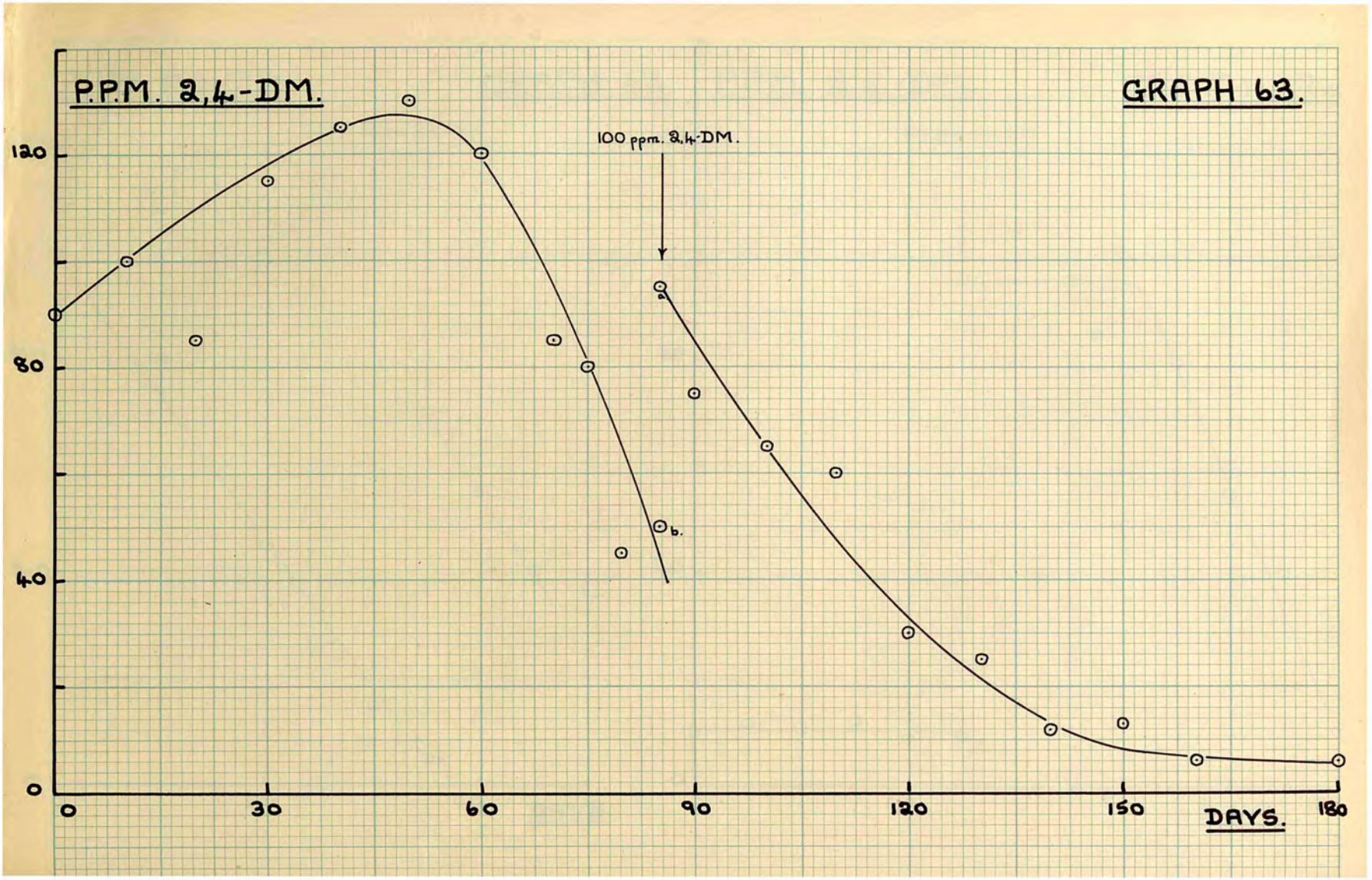
When 100 ppm. MCPA enriched perfusers were refilled directly with 10 ppm. 2,4-DM immediate and fairly rapid breakdown always ensued (Graph 60, Tables 60, 60a, 60b,). In each case there was a tendency for the breakdown rate to fall off as the perfusate toxicity approached zero. Refills at 10 ppm. were likewise broken down. Immediate switching after only one refill at 10 ppm. to a second at 100 ppm. 2,4-DM resulted in apparent loss of adaptation for no detectable breakdown occurred in the next 65 days (Table 60a,). When 100 ppm. 2,4-DM followed several changes at 10 ppm. 2,4-DM (Graph and Table 60,) breakdown commenced immediately. The rate of breakdown fell off to such an extent that it would probably have ceased altogether in about 40 to 50 days with about 30% of the original toxicity remaining.


P.P.M. MCPA OR 2,4-DCP x 0·5.
GRAPH 61.
100 ppm. MCPA.
200 ppm. 2,4-DCP.
100 ppm. 2,4-DCP.
120
80
40
0
0
50
100
150
DAYS.
a.
ba.
a1hr.
b.

MCPA followed by 2,4-dichlorphenol (2,4-DCP).

2,4-DCP at 200 ppm. was added to each of three perfusers enriched to 100 ppm. MCPA. In one of these (Table 61b,) its rate of disappearance was little more than the evaporation rate for 2,4-DCP. On returning this perfuser to 100 ppm. MCPA adaptation was found to be retained though the rate of breakdown was somewhat slower than it had been before adding the phenol. The low general activity of this perfuser probably accounts for its anomalous behaviour when compared with the other two. With these (Graph 61, Tables 61 and 61a,) the phenol disappeared very rapidly and did likewise on refilling. In one of these perfusers (Graph and Table 61,) the rate of breakdown even increased over several changes of perfusate. It is worthy of note that this behaviour is very different from that of 2,4-D followed by 2,4-DCP where the first one or two charges of 2,4-DCP disappear rapidly only to be succeeded by an apparent loss of adaptation to the phenol.


P.P.M. 2,4-DM.
GRAPH 62.
10 ppm. 2,4-DM.
12.0
8.0
4.0
0
a
b.
0
40
80
DAYS.
P.P.M. 2,4-DM.
GRAPH 62a.
100 ppm. 2,4-DM.
120
80
40
0
a.
b.
0
40
80
120
DAYS.


P.P.M. 2,4-DM.
GRAPH 63.
100 ppm. 2,4-DM.
120
80
40
0
a.
b.
0
30
60
90
120
150
180
DAYS.

2,4-dimethylphenoxyacetic acid (2,4-DM).

Muir and Hansch (85a,) reported weak growth promoting activity for this compound while Thomson et al (125,) found it to have moderate herbicidal activity. The cress-test showed it to have the fairly high Relative Toxicity of 18.

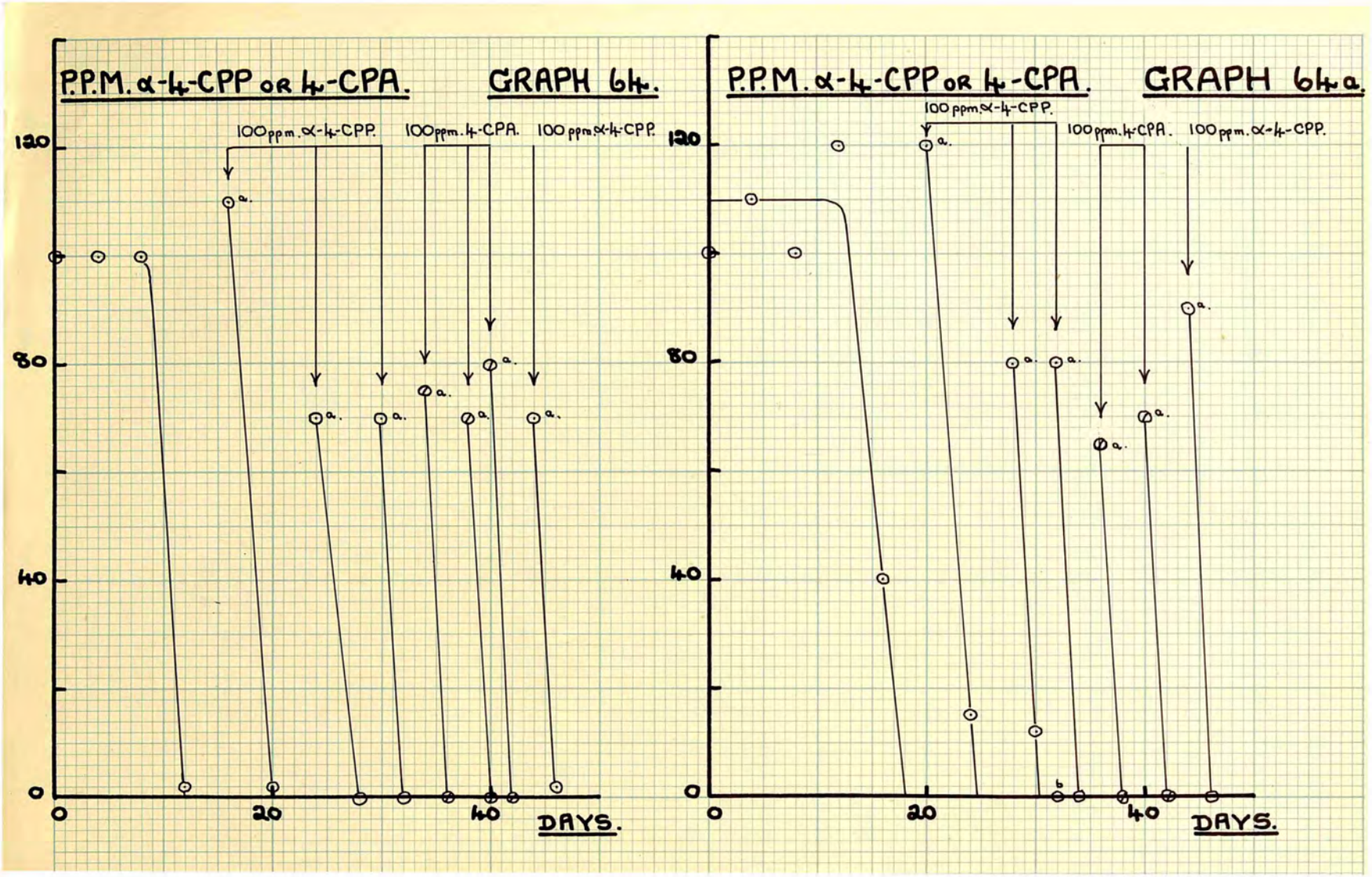
Direct Perfusion.

From the three 2,4-DM perfusions which were carried out only one consistent feature emerged - the compound disappeared from the soil in measureable time in each case. Of two perfusers started at 10 ppm. 2,4-DM, one (Graph and Table 62,) appeared to commence breakdown immediately while the other (Graph and Table 62a,) remained unaltered for 50 days. 100 ppm 2,4-DM added to this perfuser, without draining, showed a rapid rate of breakdown after a further lag of about 30 days.

At 100 ppm. 2,4-DM, one perfuser (Table 63a,) showed signs of breakdown after a lag of only 20 days while the other ((Graph and Table 63,) appeared to achieve an increase in herbicide concentration over the first 50 days, followed by a fairly rapid breakdown. The increased toxicity cannot be attributed to evaporation for this did not occur to any significant extent. It must presumeably have been due to the formation of a breakdown product more toxic to cress than 2,4-DM.

There seems to be no reason why the differing behaviour of the four 2,4-DM perfusers might not be the result of enrichment of different strains or species of micro-organisms in each case.

These could probably attack the molecule in different ways for 2,4-DM differs from 2,4-D and other halogenated phenoxy-acids in having oxidisable methyl groups which present other sites for attack on the molecule.


P.P.M. α-4-CPP OR 4-CPA. GRAPH 64.
100 ppm. α-4-CPP.
100 ppm. 4-CPA.
100 ppm α-4-CPP.
120
80
40
0
0
20
40
DAYS.
P.P.M. α-4-CPP OR 4-CPA. GRAPH 64a.
100 ppm α-4-CPP.
100 ppm. 4-CPA.
100 ppm. α-4-CPP.
120
80
40
0
0
20
40
DAYS.

α-(4-chlorophenoxy)-propionic acid (α-4-CPP).

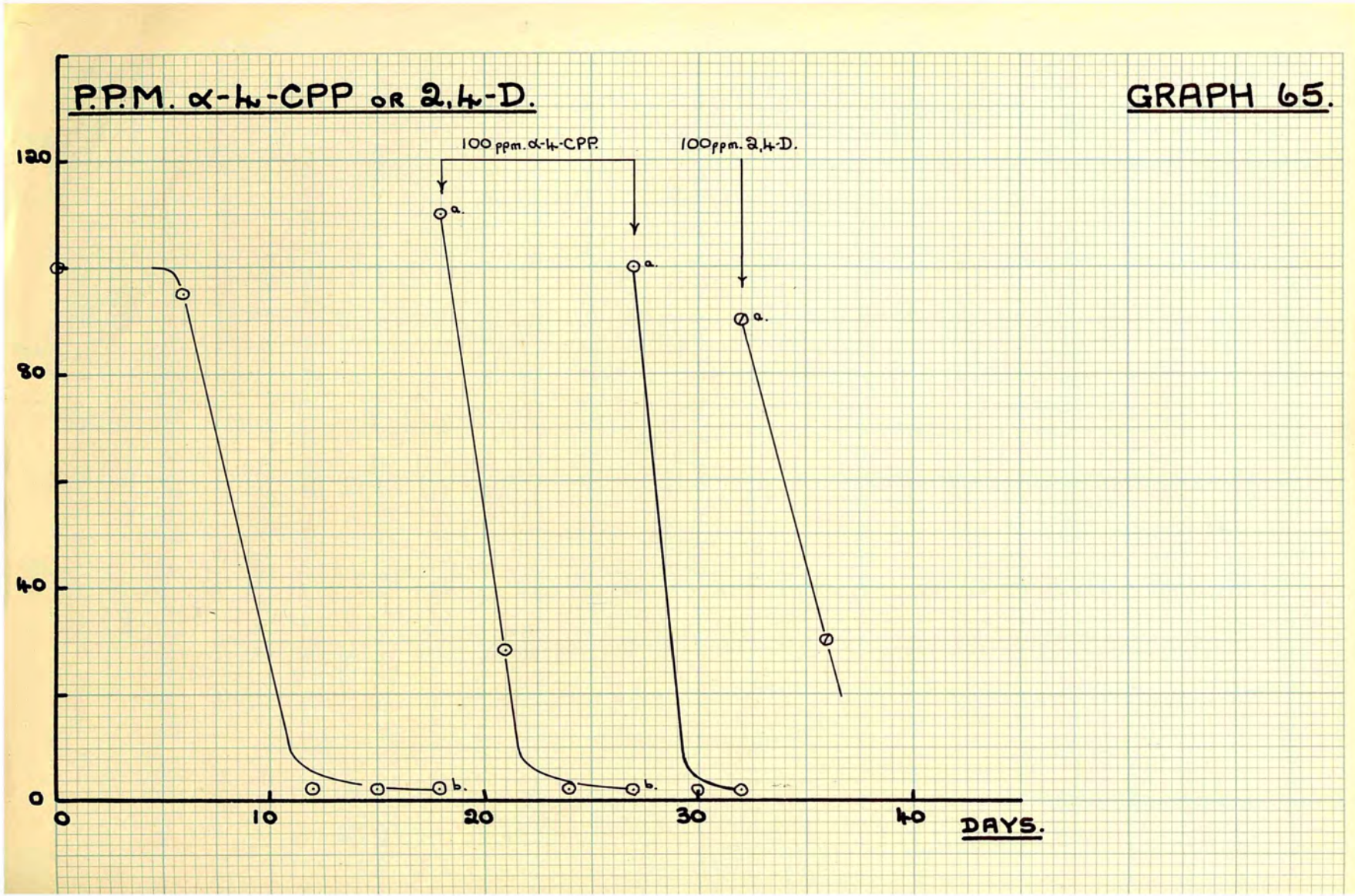
The racemic compound was found to have a very high activity in the cress-assay with a Relative Toxicity of 50.

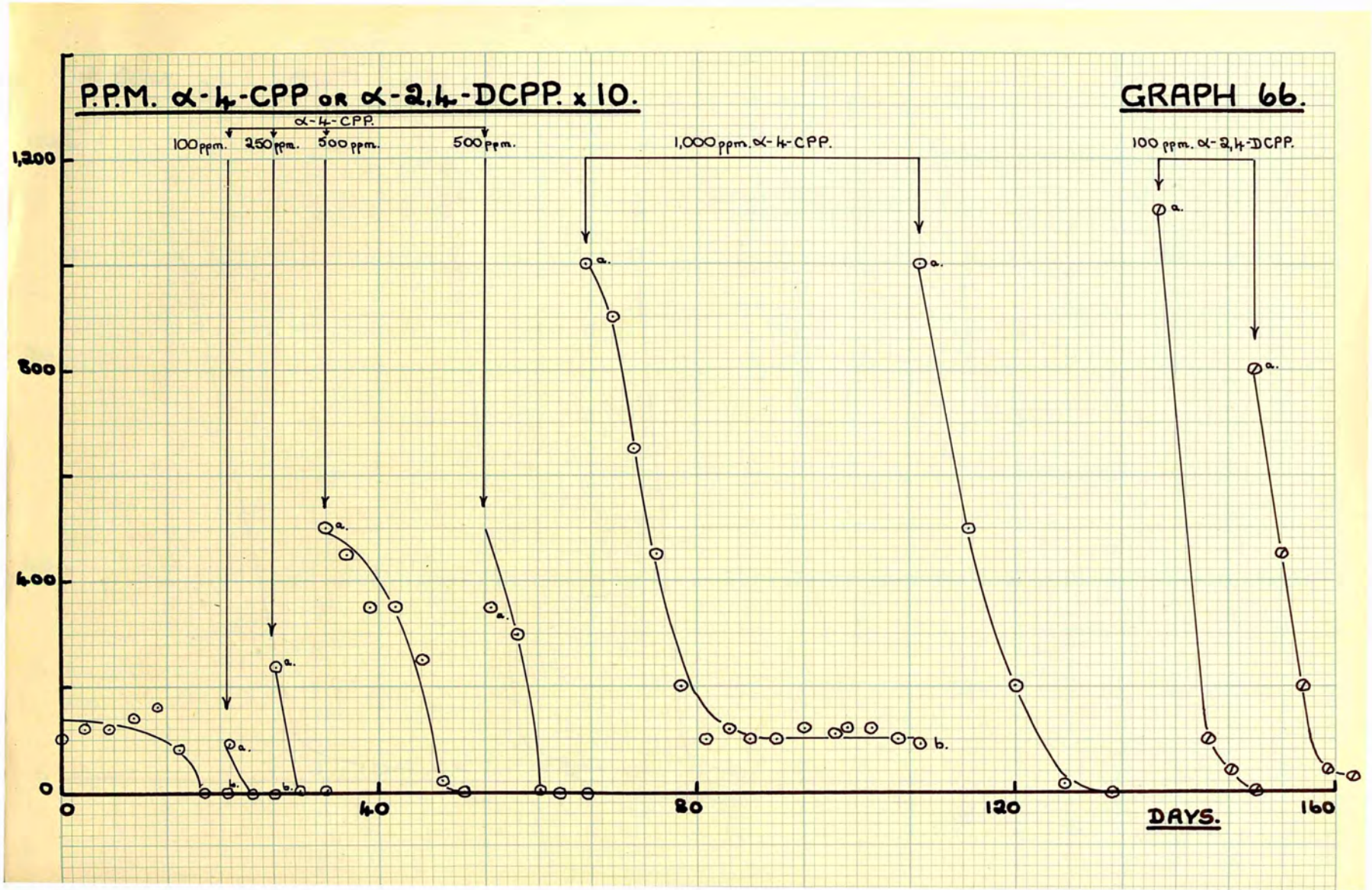
Direct Perfusion.

In each of five perfusers with 100 ppm. α-4-CPP (Graphs 64, 65, 66, Tables 64, 64a, 65, 66, 66a,) direct adaptation occurred in about 10 days, followed by a rapid and apparently complete breakdown. Subsequent refills were also rapidly broken down. In two perfusers (Graph 66, Tables 64a, 66,) there was an indication of increasing perfusate toxicity before adaptation proper and the onset of rapid post-adaptation breakdown. In those perfusers for which a sufficient number of assays were carried out before each refill (Graph 65, Tables 65, 66a,), it was found that the perfusate toxicity was not reduced to zero but to a level equivalent to about 2 to 3% of the initial value.
In one perfuser (Graph and Table 66,) higher concentrations of α-4-CPP were also broken down. At the first attempt with 1,000 ppm. α-4-CPP breakdown was rapid till the toxicity had been reduced to about 10% of the initial level. At this point it remained for a further 28 days. Complete breakdown occurred with a second refill at 1,000 ppm.

α-4-CPP followed by 4-chlorophenoxyacetic acid (4-CPA).

Rapid breakdown of 100 ppm. 4-CPA commenced as soon as it was added to a 100 ppm. enriched α-4-CPP


P.P.M. α-4-CPP OR 2,4-D.
GRAPH 65.
100 ppm. α-4-CPP
100 ppm. 2,4-D.
120
80
40
0
a.
b.
0
10
20
30
40
DAYS.


P.P.M. α-4-CPP or α-2,4-DCPP. x 10.
GRAPH 66.
α-4-CPP.
100 ppm.
250 ppm.
500 ppm.
500 ppm.
1,000 ppm. α-4-CPP.
100 ppm. α-2,4-DCPP.
1,200
800
400
0
0
40
80
120
160
DAYS.
a.
b.

perfuser (Graph 64, Tables 64, 64a,). Second and third charges behaved in the same way. Retained adaptation to ∝-4-CPP was shown by draining and refilling with this compound.

∝-4-CPP followed by 2,4-dichlorophenoxyacetic acid (2,4-D).

A perfuser (Graph and Table 65,) was enriched to 100 ppm. ∝-4-CPP and during three successive breaksdown there was a tendency for the perfusate toxicity to fall to a low steady level only. After draining, a 100 ppm. 2,4-D solution was added. Rapid loss of toxicity was immediately observed.

∝-4-CPP followed by ∝-(2,4-dichlorophenoxy)-propionic acid.

100 ppm. ∝-2,4-DCPP added to a 100 ppm. ∝-4-CPP enriched perfuser did not alter significantly in toxicity over a period of 4 days (Table 66a,). This should have been long enough for a marked loss of toxicity if simultaneous adaptation had prevailed.

When 100 ppm. ∝-2,4-DCPP was added to a 1,000 ppm. enriched ∝-4-CPP perfuser, complete breakdown did occurr though at a slow and decelerating rate (Graph and Table 66,).

These results should be compared with those obtained by adding 2,4-D to 4-CPA or ∝-4-CPP perfusers where the 2,4-D was always readily broken down.

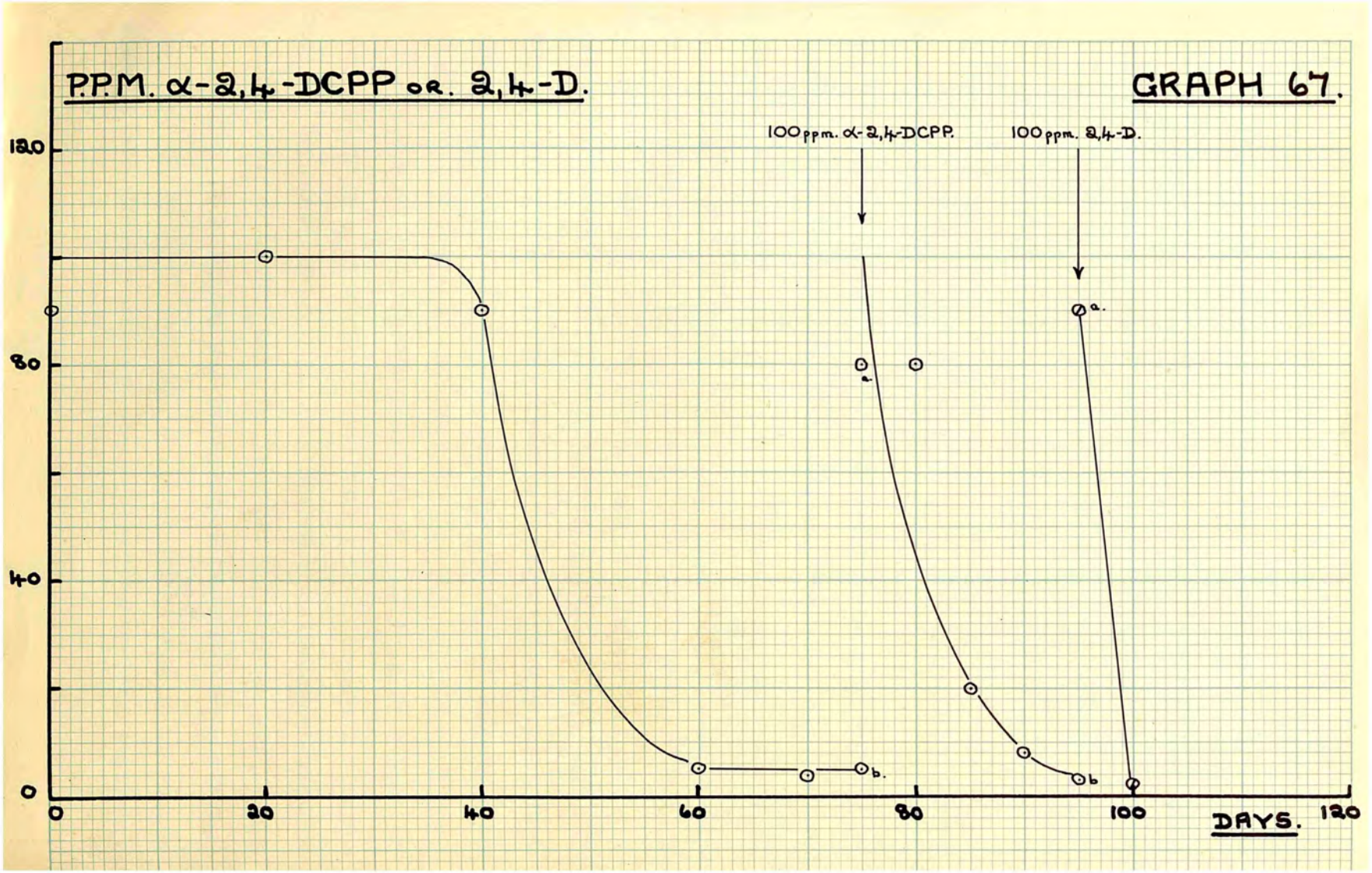
α-(2,4-dichlorophenoxy)-propionic acid (α-2,4-DCPP).

The racemic acid was shown by Synerholm and Zimmerman (120, 121,) to possess a very high physiological activity. Åberg (1a,) and Wain et al (109a, 110,) found the activity to be largely due to the (+) isomer though the (-) isomer did have a slight activity. The Relative Toxicity was found to be very high, 67.

Direct Perfusion.

Perfusion at 10 ppm. failed to produce adaptation in 80 days and, after draining and refilling at 10 ppm., not in a further 20 days (Table 67a,). At 100 ppm. adaptation occurred in 40 to 60 days. Breakdown did not go to completion but stopped when the toxicity of the perfusate had fallen to about 5% of its initial value (Graph and Table 67,). Breakdown of a 100 ppm. refill probably began with little, if any, lag and the shape of the detoxication curve suggests that a low residual level of toxicity would have been reached if perfusion had been continued long enough.

With a perfusate strength of 200 ppm. adaptation occurred in 60 days (Table 67b,). Breakdown was not very fast and ceased after a further 15 days when the toxicity of the perfusate had been reduced to about 5% of its initial value. No further change was evident in the next 20 days. After draining and refilling at 100 ppm. there was a lag of about 9 days succeeded by breakdown at about the same rate as in the immediate post-adaptative phase. Breakdown again ceased when

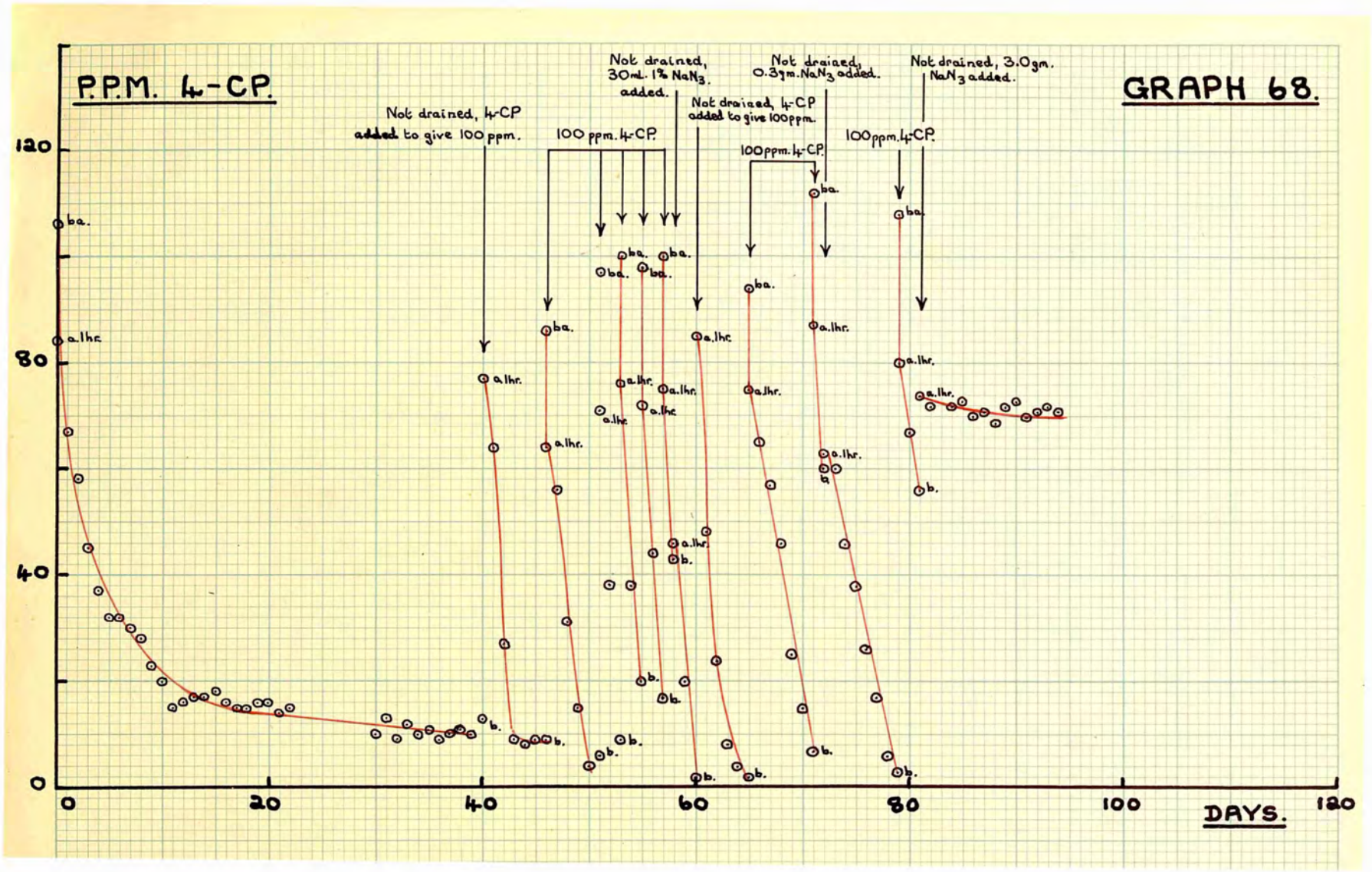

P.P.M. α-2,4-DCPP or 2,4-D.
GRAPH 67.
100 ppm. α-2,4-DCPP.
100 ppm. 2,4-D.
120
80
40
0
0
20
40
60
80
100
120
DAYS.
a.
b.

the toxicity of the perfusate was reduced to about 5% of its initial value.

The residual toxicity appeared to be a direct function of the initial perfusate concentration, suggesting that the least physiologically active enantiomorph might be resistant to bacterial degradation and responsible for the residual activity. Direct measurement of optical activity in the perfusate gave negative results which was to be expected considering the low starting concentration of racemate and small specific rotation of the separated isomers (109a,). An attempt was made to produce a larger concentration and quantity of the residual toxic agent. The perfuser (Table 67b,) was drained and refilled with 1,000 ppm. ∝-2,4-DCPP. This concentration must have proved toxic to the adapted population for no detectable breakdown occurred in 48 days.

∝-2,4-DCPP followed by 2,4-dichlorophenoxyacetic acid.

100 ppm. ∝-2,4-DCPP was added to an enriched perfuser (Graph and Table 67,). It was disappearing at a rapid rate though showing signs of slowing up to leave a residual toxicity of 5%. At this point the perfuser was again drained and refilled, this time with 100 ppm. 2,4-D. Breakdown commenced immediately and was very quickly completed.


P.P.M. 4-CP.
GRAPH 68.
Not drained, 4-CP added to give 100 ppm.
100 ppm. 4-CP.
Not drained, 30 mL. 1% NaN3. added.
Not drained, 4-CP added to give 100 ppm.
100 ppm. 4-CP.
Not drained, 0.3 gm. NaN3 added.
100 ppm. 4-CP.
Not drained, 3.0 gm. NaN3 added.
120
80
40
0
0
20
40
60
80
100
120
DAYS.
a.1hr.
ba.
b.

4-chlorphenol (4-CP).

Walker (129,) showed that 4-CP disappeared at a slow rate from soil and that the rate of disappearance was not materially affected by pretreatment of the soil with phenol or 4-CP. Pretreatment of soil with 4-CP completely prevented breakdown of 4-CPA when this was subsequently added.

Direct Perfusion.

At 100 ppm. there was no appreciable lag phase before the 4-CP started to disappear. About 20% of the phenol was quickly absorbed from solution and rapid breakdown appeared to commence immediately. Decomposition of the initial 4-CP supply tended to slow down and eventually cease when about 15 to 20% still remained (Graph 68, Tables 68, 68a, 68b, 68c,).

On adding further 4-CP, with or without draining, to give 100 ppm., rapid breakdown again commenced immediately.

It is possible that at least part of the 15 to 20% residual phenol, after the first breakdown, was not 4-CP but impurities such as 2-CP and 2,4-DCP in the technical grade material used. If not also attacked by the 4-CP adapted organisms, these substances would remain and give the perfusate a positive phenol reaction. Subsequent adaptation to such impurities may also have occurred for when one perfuser was continued for a long time (Graph and Table 68,) later batches of added phenol were broken down completely.

The results obtained with another perfuser (Table 68a,) tend to contradict the impurities theory. There, two further batches of 4-CP were added without draining the perfuser. Each time, 15 to 20% appeared to remain after a rapid breakdown phase. Impurities might be expected to accumulate reaching a final residual concentration equivalent to about 60 ppm. 4-CP.

Invoking toxic breakdown products again does not provide a completely satisfactory explanation of the observations, for each new addition of 4-CP started to breakdown immediately and at high speed, whether or not the old perfusate was drained before recharging.

Disappearance of 4-CP from the perfusers cannot be solely attributed to evaporation for in the presence of a toxic sodium azide concentration (Table 68c,) the rate of disappearance was very slow.

A theory can be derived which more closely fits the observations regarding the variation in breakdown rate and the residual phenol. The first stage of breakdown proper may be the limiting reaction following a reversible absorbtion of the 4-CP onto an enzyme or other active surface, the degree of absorbtion depending on the 4-CP concentration. During early post-adaptation stages, the 4-CP/active surface affinity may be low due to incomplete adaptation and so, as the 4-CP concentration falls, the degree of absorbtion and rate of the first breakdown reaction also falls to a very low level. As the adaptation became more"fixed", breakdown

would continue at a measureable rate down to much lower 4-CP concentrations, even appearing to go to completion.

0.1% sodium azide tended to slow down the rate of 4-CP breakdown but could not stop it,(Graph 68, Tables 68, 68c,). Breakdown was completely inhibited by 1% sodium azide (Graph and Table 68,). It is probable that the 4-CP breakdown chain does not involve a normal biological oxidation reaction for these are usually inhibited by much lower azide concentrations.

2,4-dichlorphenol (2,4-DCP).

Direct Perfusion.

About 20% of the 2,4-DCP was very quickly absorbed from the perfusate (most probably by the soil) when added to the perfuser at any of the concentrations used. Following this, the behaviour was somewhat dependent upon concentration. With an initial concentration of 100 ppm. or less (Graphs 70, 71, 72, 72d, Tables 70, 71, 71a, 72, 72a, 72d,) the rapid absorbtion was followed by a period of several days during which the 2,4-DCP disappeared at a slow steady rate. When the concentration had fallen to about 10 ppm. the reaction ceased and the perfusing concentration remained steady until the addition of more 2,4-DCP. The second batch of phenol similarly passed through the absorbtion phase but rapid and complete decomposition then set in. Subsequent batches of 2,4-DCP followed the same general course as the second one. The perfusing 2,4-DCP concentration could be slowly stepped up without producing aberrant behaviour.

When the initial perfusate concentration was of 200 ppm. or more of 2,4-DCP, the rapid absorbtion was followed by a slow fall in concentration at a rate little more than could be expected from evaporation alone (Tables 72b, 72c, 72e,). There was, again, a tendency to attain a steady low concentration. Refilling without draining caused a repetition of the process though the final steady concentration was higher. A further refill produced the same results with an even higher steady concentration.

2,4-dichlorphenol (2,4-DCP).

Direct Perfusion.

About 20% of the 2,4-DCP was very quickly absorbed from the perfusate (most probably by the soil) when added to the perfuser at any of the concentrations used. Following this, the behaviour was somewhat dependent upon concentration. With an initial concentration of 100 ppm. or less (Graphs 70, 71, 72, 72d, Tables 70, 71, 71a, 72, 72a, 72d,) the rapid absorbtion was followed by a period of several days during which the 2,4-DCP disappeared at a slow steady rate. When the concentration had fallen to about 10 ppm. the reaction ceased and the perfusing concentration remained steady until the addition of more 2,4-DCP. The second batch of phenol similarly passed through the absorbtion phase but rapid and complete decomposition then set in. Subsequent batches of 2,4-DCP followed the same general course as the second one. The perfusing 2,4-DCP concentration could be slowly stepped up without producing aberrant behaviour.

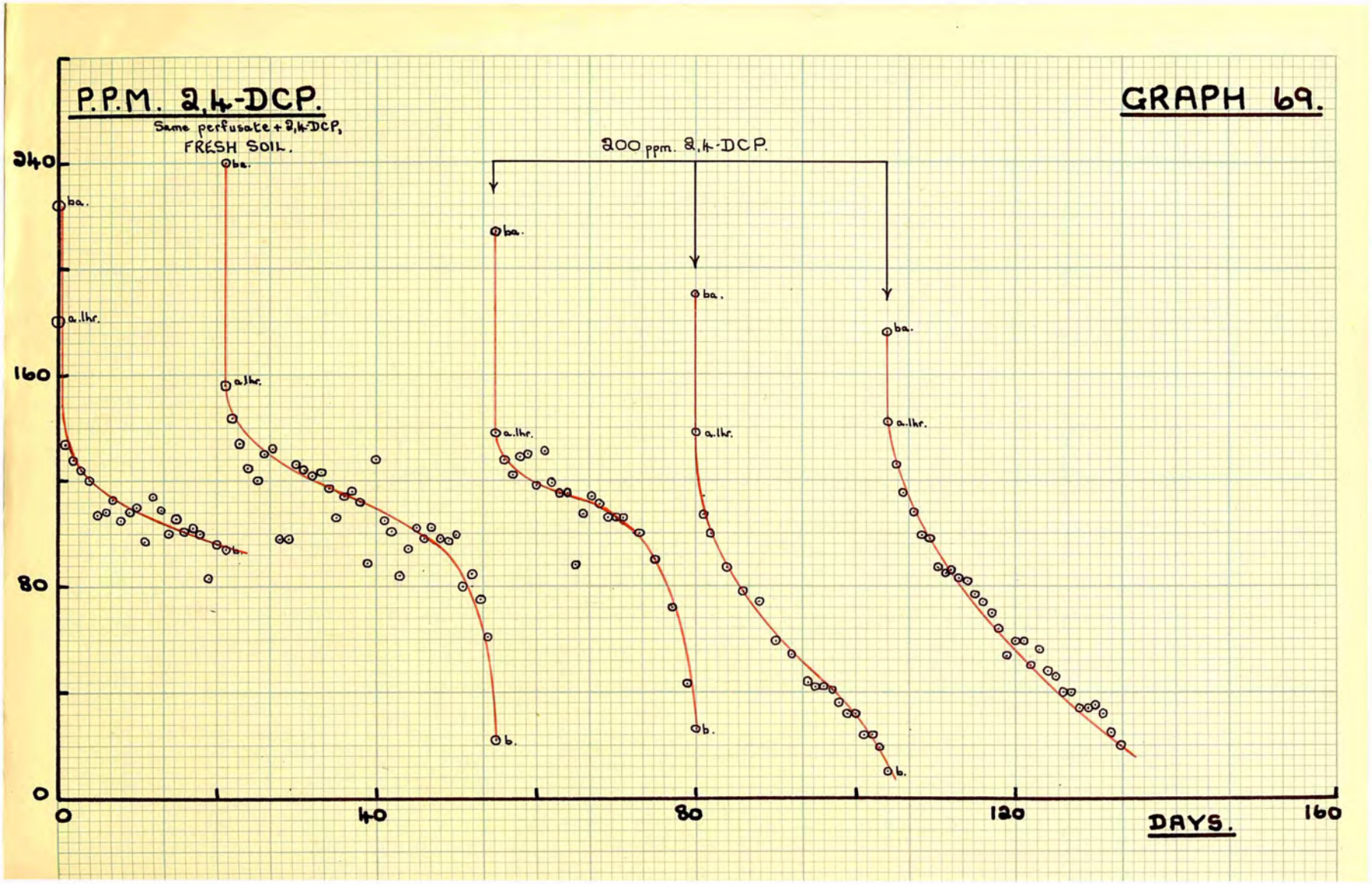
When the initial perfusate concentration was of 200 ppm. or more of 2,4-DCP, the rapid absorbtion was followed by a slow fall in concentration at a rate little more than could be expected from evaporation alone (Tables 72b, 72c, 72e,). There was, again, a tendency to attain a steady low concentration. Refilling without draining caused a repetition of the process though the final steady concentration was higher. A further refill produced the same results with an even higher steady concentration.

Eventually, after about 60 days, the 2,4-DCP concentration fell abruptly to zero. From this point further replenishments, without draining, disappeared at ever increasing rates. Behaviour was slightly different when, after reaching the steady state, the perfuser was drained before refilling at 200 ppm. 2,4-DCP (Tables 72b, 72c,). The absorbtion and slow breakdown phases were repeated but, after about 30 days, the concentration fell rapidly to zero. Draining and refilling at 200 ppm. was again followed by the rapid absorbtion and slow breakdown phases, succeeded by the onset of a rapid fall in phenol concentration. After further refills, fairly active breakdown followed immediately after the initial absorbtion phase though the rate of breakdown was never as high as in a perfuser adapted initially at 100 ppm. 2,4-DCP.

Several workers have found bacteria,from soil and other sources, readily adaptable to phenol (14, 22, 30, 31, 40, 46, 105, 108, 109, 114, 115, 116, 129,) while Walker (129,) found 2-chlorphenol to be quickly decomposed in soil. During the present research 4-chlorphenol has also been shown to be fairly labile in soil. Consequently there seems to be no obvious reason why adaptation to 2,4-DCP should prove to be such a difficult process. An explanation must be sought in the toxicity of the substance for 2,4-DCP has been shown to be a very potent bactericidal agent (79, 94,). The production of an enriched perfuser population probably results from a compromise between adaptation and multiplication of the adapted cells on the one hand and the bactericidal and

bacteriostatic action of the 2,4-DCP on the other. It is highly probable that the 2,4-DCP also acts adversely on the enzymic proteins taking part in the adaptation reaction. With the higher 2,4-DCP concentrations used, a major portion of the initial soil population was probably killed quickly (few colonies were ever obtained when isolates were made from any perfuser onto 2,4-DCP agar). Though adaptation may have occurred readily in the residual phenol resistant cells, cell division may have been severely restricted and hardly able to keep pace with the prevailing death rate. The 2,4-DCP concentration would be lowered slowly by this small active population and, in turn, the lower phenol concentration would lower the death rate and permit increased cell multiplication. (98,). Together, these factors constitute a type of " auto-catalytic system " which, under favourable circumstances would result in an ever increasing rate of 2,4-DCP breakdown. and curves of the type found (Graph 69, Tables 69, 72b, 72c,). Refilling at a higher concentration would, to some extent, reduce the population as before and the cycle of changes would be repeated. It is to be expected that a population with a high 2,4-DCP tolerance would eventually develop, able to break down the phenol at a fairly high rate limited by factors other than population size.

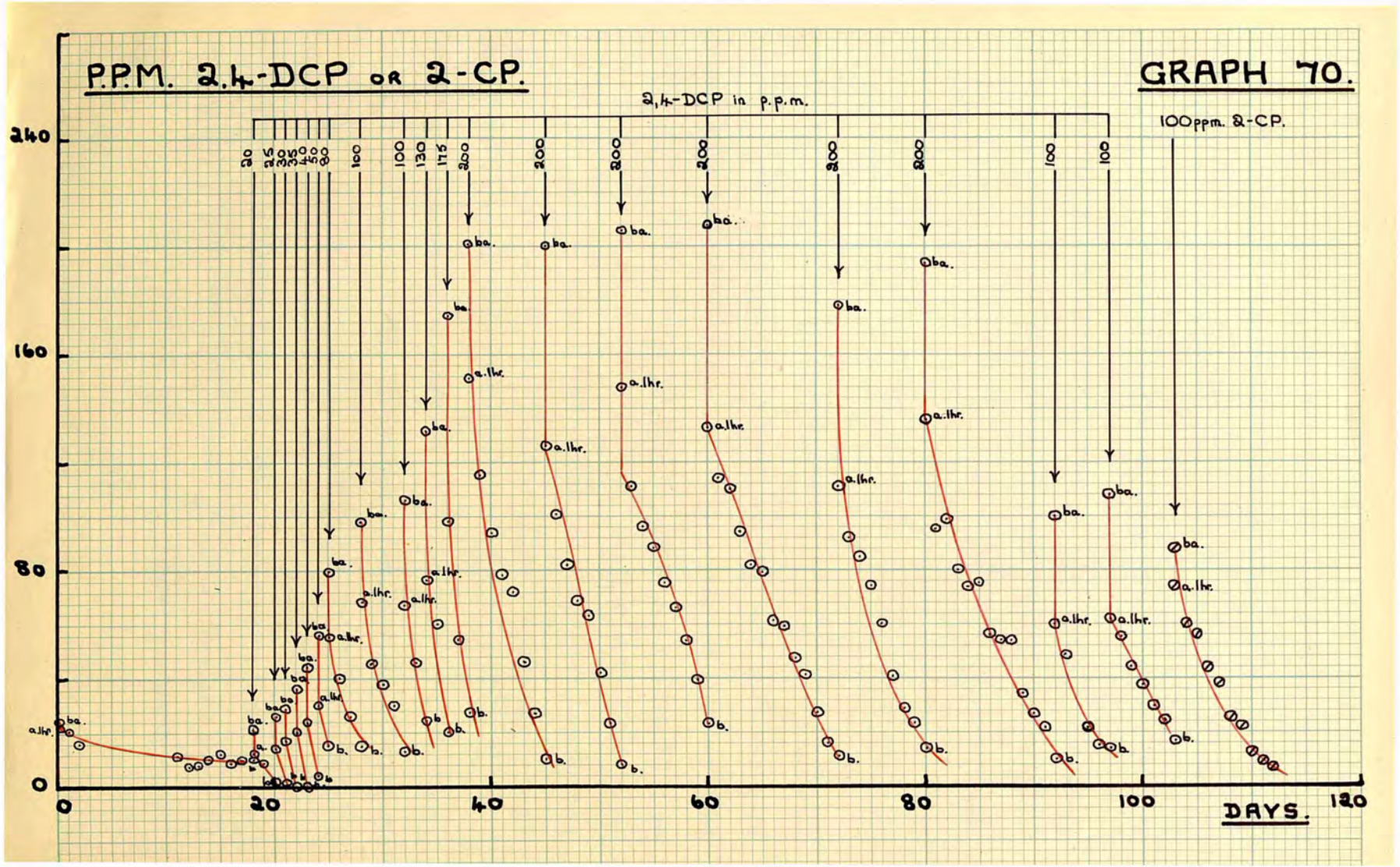
It is probable that behavior at other 2,4-DCP concentrations was also a function of concentration but interpretation of the results is complicated by the simultaneous existance of the equilibrium between 2,4-DCP in the perfusate and 2,4-DCP adsorbed on the soil and other surfaces.

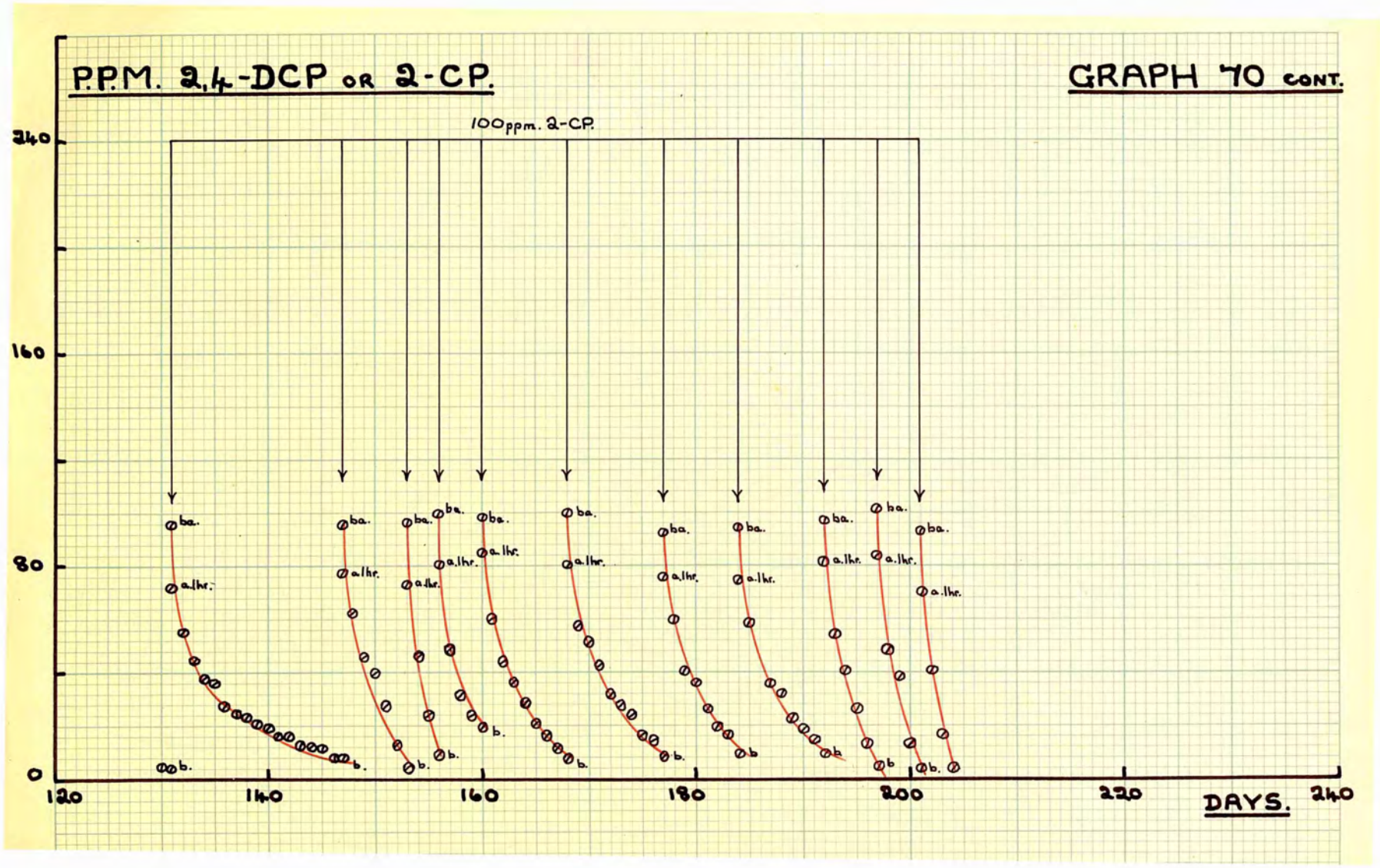

P.P.M. 2,4-DCP.
GRAPH 69.
Same perfusate + 2,4-DCP, FRESH SOIL.
200 ppm. 2,4-DCP.
240
160
80
0
0
40
80
120
160
DAYS.
ba.
a.1hr.
b.

It was noted with several 2,4-DCP perfusers that the perfusate tended to aquire a faint orange-pink colouration, and in one case a faint purple-brown, when 2,4-DCP was disappearing at a fast rate. The perfusates became virtually colourless again when most of the phenol had gone. This behaviour was observed at both early and late stages in the life of a perfuser and suggests the formation of quinonoid or other highly conjugated intermediates in the breakdown chain.

Assisted enrichment (Transferred adaptation).

Two attempts were made to accelerate the enrichment of a perfuser but, as they involved the same perfusate, the results have been combined (Graph and Table 69,). Perfusate was taken from a 2,4-DCP perfuser, in an active breakdown phase, and added to fresh soil. The typical initial absorbtion was followed by a very slow disappearance phase. The perfusate was therefore removed, fortified with further 2,4-DCP and reperfused through another batch of soil. The behaviour pattern was, at first, repeated but followed after 30 days by a rapid breakdown to a negligable concentration. Draining and refilling produced the same effect but with a shorter slow-disappearance phase. With two subsequent refills the initial absorbtion was succeeded by a moderate breakdown rate. As this rate was only a fraction of that following direct adaptation, enriched perfusers were never produced other than by the direct method.


P.P.M. 2.4-DCP OR 2-CP.
GRAPH 70.
2,4-DCP in p.p.m.
100ppm. 2-CP.
240
160
80
0
0
20
40
60
80
100
120
DAYS.
30
25
30
35
40
50
80
100
100
130
175
200
200
200
200
200
200
100
100
ba.
a.1hr.
b.


P.P.M. 2,4-DCP OR 2-CP.
GRAPH 70 CONT.
100ppm. 2-CP.
240
160
80
0
120
140
160
180
200
220
240
DAYS.
ba.
a.1hr.
b.

Effect of inhibiters.

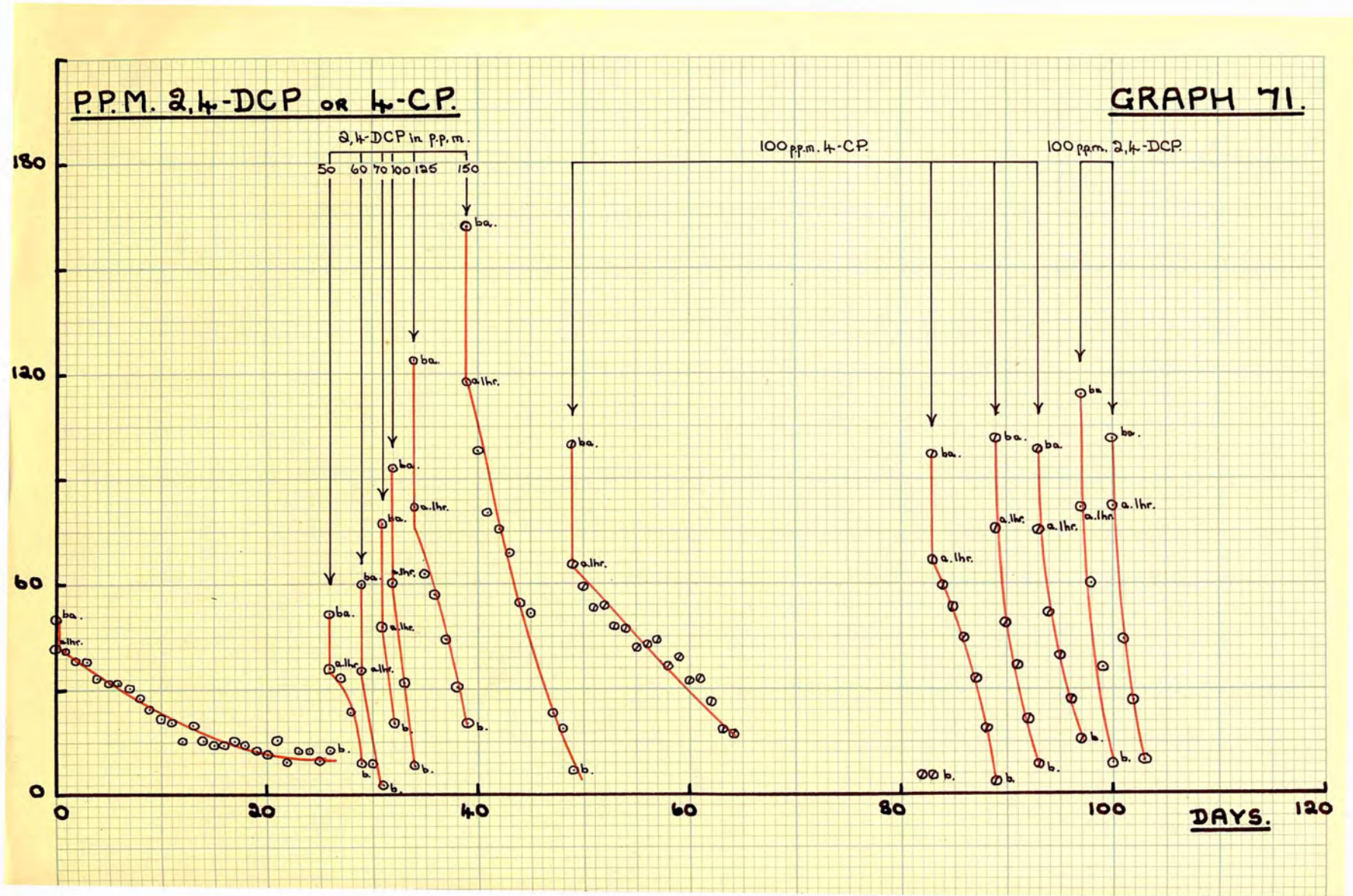
Sodium azide was the only inhibiter tried in a 2,4-DCP perfuser. 200 ppm. seemed to completely inhibit biological breakdown and lower the disappearance rate to a value consistent with the evaporation rate. The result is, however, not strictly valid as the perfuser had been adapted initially to 2,4-D.(Graph and Table 41,).

2,4-DCP followed by 2-CP. (2-chlorphenol).

Initial adaptation to 20 ppm. 2,4-DCP occurred readily and the perfusing concentration was built up gradually, over a number of refills with drainage, till 200 ppm. 2,4-DCP was being dealt with successfully. The concentration was dropped to 100 ppm. for two successive changes them switched, after draining, to 100 ppm. 2-CP. (Graph and Table 70,). Breakdown commenced immediately and continued rapidly till only about 6 ppm. remained, at which level it stayed for a further 20 days. The perfuser was drained and refilled a further twelve times with 100 ppm. 2-CP. Each time the phenol disappeared quickly from the perfusate.

2,4-DCP followed by 4-chlorphenol (4-CP).

After adaptation at 50 ppm. perfusate concentrations of 2,4-DCP were built up in stages to 150 ppm. (Graph and Table 71,) and 200 ppm. (Table 71a,). Both perfusers were then drained and refilled with 4-CP, at 100 and 150 ppm. respectively. In both cases


P.P.M. 2,4-DCP OR 4-CP.
GRAPH 71.
2,4-DCP in p.p.m.
50 60 70 100 125 150
100 p.p.m. 4-CP.
100 p.p.m. 2,4-DCP.
ba.
a.1hr.
b.
180
120
60
0
0
20
40
60
80
100
120
DAYS.

the immediate partial absorbtion was followed by a short period of apparently rapid breakdown and then by a long slow disappearance typical of a primary adaptation to 4-CP. One of the perfusers (Graph and Table 71,) was drained and refilled with 100 ppm. 4-CP which disappeared rapidly from the perfusate. A further two refills of 4-CP were similarly broken down.

The results suggest that adaptation to 2,4-DCP does not involve simultaneous adaptation to 4-CP but that a normal adaptation to this second compound occurrs.during the perfusion.

Rapid breakdown of 2,4-DCP on returning the perfuser to this compound showed that the intermediate 4-CP adaptation had not broken the primary 2,4-DCP adaptation.

2,4-DCP followed by 2,4-dichlorophenoxyacetic acid (2,4-D)

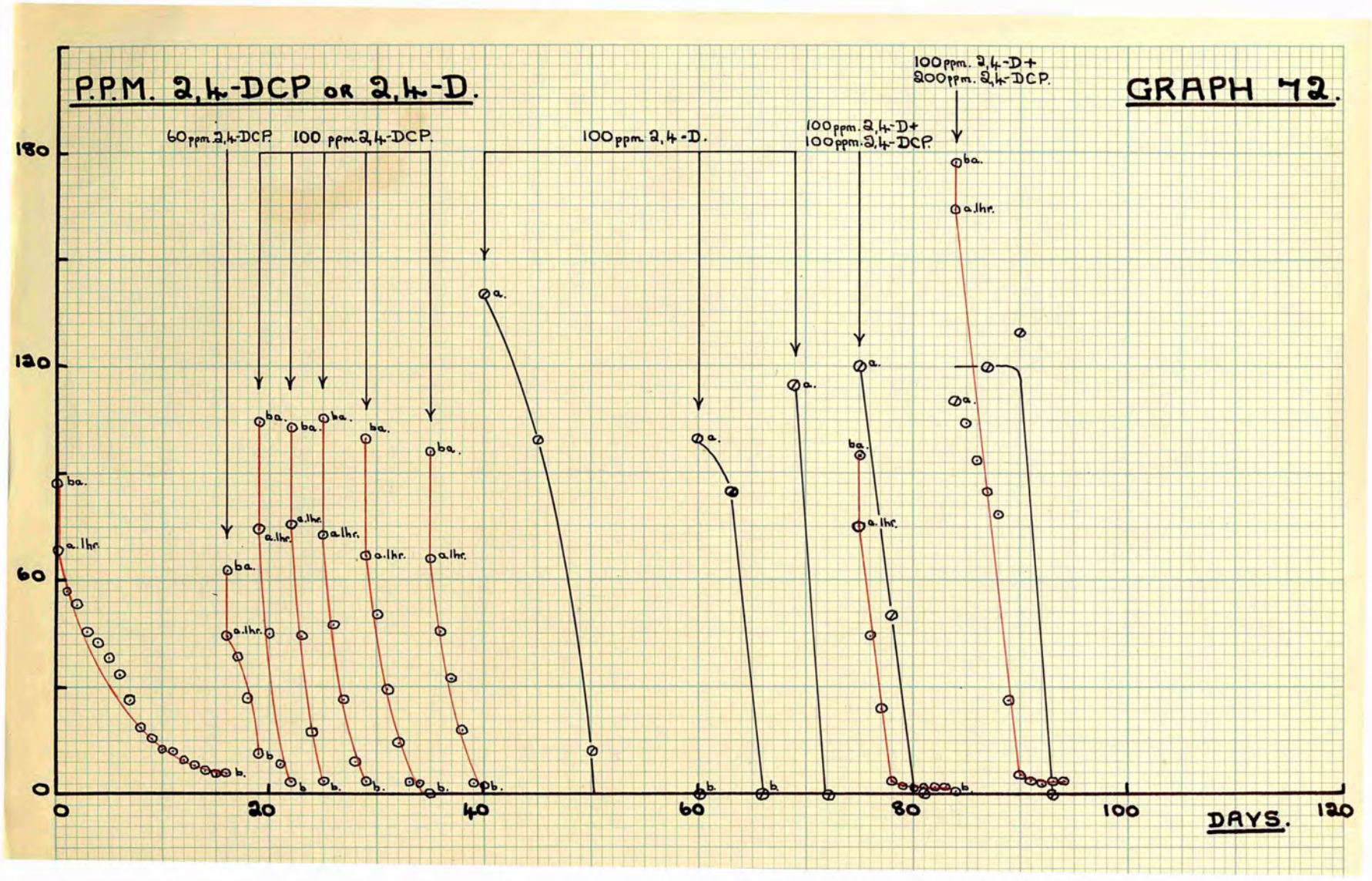
As 2,4-DCP seemed to be a likely intermediate in the 2,4-D breakdown chain, several perfusers adapted to 2,4-DCP were switched to 2,4-D to see whether adaptation to 2,4-DCP had any effect on subsequent adaptation to 2,4-D. Inconclusive results were obtained on adding 100 ppm. 2,4-D to perfusers which had been enriched to 100 or 200 ppm. 2,4-DCP. In three cases (Graph 72, Tables 72, 72a, 72b,) delays of only five days (much shorter than the normal 2,4-D lag) suggested that simultaneous adaptation was operative. In two other perfusions (Graph 72d, Tables 72c, 72d,) delays of 10 and 12 days, respectively, were observed. These values do not differ significantly from the normal lag period for

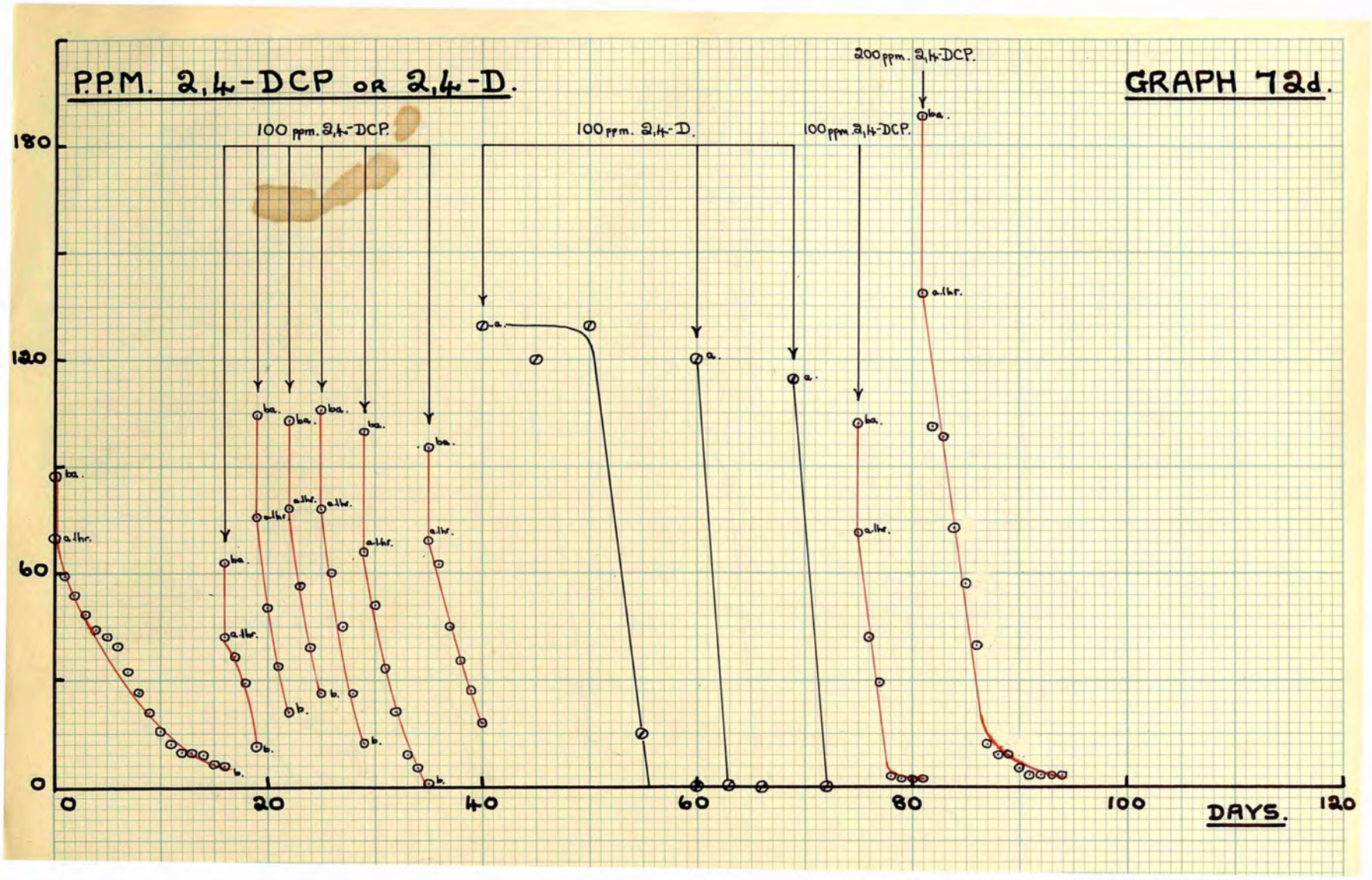
100 ppm. 2,4-D.

Two perfusers (Tables 72b, 72c,) were further switched from 100 to 1,000 ppm. 2,4-D. The newly aquired 2,4-D adaptation appeared to be destroyed. Such behaviour had already been observed when the concentration was increased too rapidly in purely 2,4-D enriched perfusers.

After several refills with 100 ppm. 2,4-D, one perfuser (Graph and Table 72d,) was returned to 2,4-DCP. This was readily broken down at both 100 and 200 ppm. It can be assumed that 2,4-DCP adaptation had been retained despite the 2,4-D treatment.

When 1,000 ppm. 2,4-D was added to a perfuser enriched to 100 ppm. 2,4-DCP (Table 72e,), twenty four days elapsed before rapid breakdown set in. This is of the same order as the normal lag for 2,4-D at 1,000 ppm. After several recharges at high 2,4-D concentrations, the perfuser was returned to 2,4-DCP at 100 and then 200 ppm. Adaptation was found to be retained. 400 ppm. 2,4-DCP produced a temporary inhibition of breakdown similar to that experienced with directly adapted 2,4-DCP perfusers at higher concentrations. Rapid breakdown was maintained on returning to lower concentrations. The perfusate concentration was then built up in stages till 600 ppm. was tried. Again, temporary inhibition returned and breakdown continued at a slow rate till the concentration had fallen to about 100 ppm. when rapid decomposition again set in.


P.P.M. 2,4-DCP OR 2,4-D.
GRAPH 72.
60 ppm. 2,4-DCP.
100 ppm. 2,4-DCP.
100 ppm. 2,4-D.
100 ppm. 2,4-D+
100 ppm. 2,4-DCP.
100 ppm. 2,4-D+
200 ppm. 2,4-DCP.
180
120
60
0
0
20
40
60
80
100
120
DAYS.
ba.
a.1hr.
a.
b.


P.P.M. 2,4-DCP OR 2,4-D.
GRAPH 72d.
100 ppm. 2,4-DCP.
100 ppm. 2,4-D.
100 ppm. 2,4-DCP.
200 ppm. 2,4-DCP.
180
120
60
0
0
20
40
60
80
100
120
DAYS.
ba.
a.
a.thr.
b.

2,4-DCP followed by 2,4-D and later by 2,4-DCP/2,4-D mixture.

After normal adaptation and enrichment to 100 ppm. 2,4-DCP (Graph 72, Tables 72, 72a,) the perfusers were switched to 100 ppm. 2,4-D. Breakdown at a moderate rate commenced after a lapse of about 5 days. Further refills at 100 ppm. 2,4-D went at a slightly higher rate. One perfuser (Graph and Table 72,) was refilled with a mixed solution containing 100 ppm. 2,4-D and 100 ppm. 2,4-DCP. Both were broken down at a high rate with no evidence of mutual interference. For the next refill the mixture was changed to 100 ppm. 2,4-D / 200 ppm. 2,4-DCP. This time there was an immediate lag in 2,4-D breakdown and a partial inhibition of 2,4-DCP breakdown following an initial rapid fall in concentration. The 2,4-DCP breakdown soon recovered and when, after a further rapid fall, the phenol concentration was reduced to a negligable level, the 2,4-D concentration also dropped quickly to zero.

With the other perfuser (Table 72a,) the intermediate 2,4-D perfusions were followed immediately by a mixture containing 100 ppm. 2,4-D and 200 ppm. 2,4-DCP. After the initial rapid fall in 2,4-DCP concentration, the rate of breakdown decreased for about 10 days and then accelerated, quickly reducing the 2,4-DCP concentration to zero. During this time the 2,4-D concentration had remained constant at the initial level. Within a few days of disappearance of the 2,4-DCP a rapid breakdown set in and the 2,4-D level was also reduced to zero. Disappearance of all toxicity within 3 days of adding 100 ppm. 2,4-D showed that active enrichment to this

compound had been maintained.

The results suggest that adaptation and enrichment to 2,4-DCP does not involve simultaneous adaptation to 2,4-D but that the same population is capable of adaptation, independently, to both compounds. The population developed in response to 2,4-DCP perfusion soon converts to a 2,4-D enriched one when subjected to the action of this compound. It may be that the enzyme systems required by the two compounds are so similar that interconversion can occur.

If 2,4-DCP is an intermediate in 2,4-D breakdown, it would only necessitate the elaboration of enzymes required to form 2,4-DCP from 2,4-D in order to convert organisms active against 2,4-DCP into those active against 2,4-D as well.

It is possible to explain the inhibiting action of 200 ppm. 2,4-DCP on 2,4-D breakdown (Graph 72, Tables 72, 72a,) along two different lines.

(1). If 2,4-DCP is an intermediate in 2,4-D breakdown. It is to be expected that the 2,4-D would remain virtually untouched till the 2,4-DCP level had been reduced to a low level consistamt with that normally obtaining in the chain of, presumably reversable, reactions bringing about 2,4-D degradation when alone at 100 ppm. The results with these two perfusers suggest that an equilibrium level of less than 5 ppm. 2,4-DCP must operate for 2,4-D breakdown did not commence till this 2,4-DCP level had been reached. This level agrees with the observation that during the active breakdown of 2,4-D in purely 2,4-D perfusers, phenol reactions equivalent to more than 5 ppm. 2,4-DCP were rarely found.

The behaviour of a mixture of 100 ppm. each of 2,4-DCP and 2,4-D (Graph and Table 72,), where each was broken down apparently independently, suggests that 2,4-DCP is not an intermediate in the 2,4-D breakdown chain. Rather, it would seem that both compounds can be broken down independently and simultaneously by the same organisms or, at least, by a common mixed population.

(2). If 2,4-DCP is not an intermediate in 2,4-D breakdown. The most likely explanation of the 2,4-DCP inhibition would then lie in its toxicity. During the enrichment phases of perfusers containing 2,4-DCP alone, 100 ppm. was found to be much less toxic than 200 ppm. In mixture with 2,4-D, the inhibiting action of 200 ppm. 2,4-DCP may therefore be accounted for by either:

(a). reduction in numbers and general vigour of the organisms attacking both compounds, or

(b). selective inhibition of the enzyme system acting on 2,4-D

Action of type (a) may be further subdivided:

(i). inhibition of the 2,4-D organism in a mixed active population, or

(ii). inhibition of a uniform population attacking both compounds.

Type (b) activity may also be subdivided:

(iii). inhibition of the 2,4-D enzyme system, and probably others as well, by differential protein denaturation.

(iv). selective inhibition of the 2,4-D breakdown enzyme by competitive absorbtion resulting from structural similarity.

DISCUSSION.

I. The nature of the detoxication process.

Non-biological breakdown, in soil, of aromatic compounds including phenols (105,) and non-biological oxidation of other organic compounds (36,) has been shown to occur. Disappearance of herbicides from soil may be partly accounted for in this way, particularly the slow disappearance of compounds such as 2,5-D which show no true adaptative phenomena. However, many workers (1, 3, 7, 8, 9, 16, 28, 42, 43, 47, 48, 56, 57, 57a, 59, 62, 77, 87, 88, 89, 90, 92,) have concluded that the disappearance of selective herbicides, of the phenoxyacetic type, from soil results largely from biological activity. Newman and Thomas (89,) claim to have produced crude cultures, from pretreated soils, which possessed such detoxicating action. Audus (8, 9,) showed that pure cultures of Bacterium globiforme, isolated from enriched soils, could readily break down 2,4-D. Jensen and Petersen (56,) isolated two organisms, one resembling B,globiforme, which could decompose 2,4-D and MCPA and the other, Flavobacterium aquatile which could only break down 2,4-D. On the other hand, Anderson and Baker (3,) failed to achieve breakdown of 2,4-D by micro-organisms in culture media. During the present investigation many attempts to produce 2,4-D breakdown by pure cultures of B.globiforme, isolated from enriched perfusers, all resulted in failure. It was possible to detect breakdown of 4-CPA, 2,4-D and MCPA in crude, though soil-free, bacterial suspensions produced from similar active perfusers (Graphs 19, 20, 29a, 49,

Tables 19, 20, 29a, 29b, 49,). Breakdown of these compounds was also accomplished by perfusing them as suspensions over sterile, acid-washed pot. The results suggest that while B.globiforme may be largely responsible for the breakdown of 2,4-D and other herbicides in soil, it cannot function alone. The part played by the other soil organisms was not determined though there are several possibilities, eg. supplying essential metabolites to a demanding B.globiforme, or removing breakdown products toxic to,and unattacked by, B.globiforme.

Of the three criteria of biological action (Walker, 129,) namely,

(1). Presence of a lag phase during which adaptation or selection occurrs.

(2). Loss of activity by sterilisation.

(3). Loss of activity in the presence of toxic agents,

(1) and (3) have been shown directly and (2) by an indirect process to apply to the breakdown of 2,4-D and related herbicides in soil perfusers.

(1).Presence of a lag phase. All compounds,which were eventually broken down actively in soil perfusers, exhibited an initial lag phase during which there was little or no sign of activity. The lag phase was usually followed by a period of rapid breakdown to zero concentration. In the perfusion of 2,4-D there was a very rapid transition from the non-active pre-adaptation state to the stage of rapid

breakdown. The rates of breakdown were so high that very large populations must have been operating. To produce such large populations in no more than two days from single mutant cells is in direct opposition to the extremely slow growth of B.globiforme in pure cultures. Either adapted cells can grow and divide more readily in soil than in culture media or, the enriched population is not produced from a single adapted cell but rather by simultaneous adaptation of all the cells in an existing population. Both alternatives are not unlikely.

Further additions of the same substrate were usually broken down with no further measureable lag. Some exceptions to this general behaviour were observed. Perfusate concentration was the commonest cause of aberration. 3-CPA was not broken down completely at 100 ppm. but behaved normally at 10 ppm. α-2,4-DCPP behaved in the opposite way in that adaptation appeared to occur at 100 ppm. but not at 10 ppm. As with α-4-CPP breakdown did not go to completion. The effect of concentration on the adaptation process cannot be deduced from the results obtained for it was possible to adapt to 2,4-D at 10, 100 and even 1,000 ppm. though adaptation at this high concentration was somewhat erratic. Also, on refilling at this high concentration there was sometimes an apparent loss of adaptation. MCPA enriched perfusers also showed a tendency to further short lag periods on refilling. It is most probable that these secondary lags resulted from intoxication of immature adapted cells before the adaptation enzyme(s) had become stabilised.

The phenols 4-CP and 2,4-DCP appeared to reverse

the normal adaptation phenomena. There was a marked fall in concentration as soon as the phenol was added to the perfuser. After a day or so the rate of disappearance decelerated and remained low for varying periods of time before a final acceleration and fall to zero concentration. The behaviour pattern was repeated with each refill, the inactive middle phase being shorter each time. Eventually it became non-existant and initial and final rapid disappearance phases became continuous. The initial disappearance phases were probably non-biological functions, being compounded from evaporation, adsorbtion and chemical inactivation. The middle phase was probably one of evaporation and inactivation while true biological action was responsible for the final active phase. The gradual elimination of the middle phase was probably a measure of the development of phenol resistance and not of adaptation. That the above is probably a true interpretation of the observations is supported by the following, (i). the initial rapid drop in concentration was roughly proportional to the initial concentration, (ii). the intermediate lag phases could be eliminated by starting at a low phenol concentration and building up gradually with each refill, (iii). intermediate lag phases could be induced in such systems by adding a high enough phenol concentration.

(2). Loss of activity by sterilisation. This is usually demonstrated in one of two ways, (a). breakdown and adaptation are not observed in sterilised soil, (b). breakdown is terminated by sterilising an active soil. During the present

experiments a third method was employed. It had been found that adaptation to any of the labile phenoxyacids could be transferred to fresh soil by perfusing it with spent perfusate from an active perfuser. Further enriched perfusers were readily obtainable in this way. The transfer of activity could be due to one of two things, (i). carry over of adapted organisms from the enriched to the fresh soil, or (ii). stimulation of an existing population by some growth factor or breakdown product in the spent perfusate. Heat sterilised spent perfusate (Graph and Table 50a,) was unable to initiate breakdown in fresh soil. Although this evidence favours proposal (i) and, by inference, the assumption that breakdown is by an adapted biological system, the possibility cannot be completely excluded that the transfer reaction was due to the carry over in spent perfusate of a thermolabile factor resulting from the previous breakdown.

(3). Loss of activity in the presence of toxic agents.

The self inhibiting action of some compounds at high concentrations and inhibition by related resistant compounds are best considered later under the appropriate headings rather than under the heading of toxic agents. This leaves the results obtained with the common respiration inhibiters sodium azide and sodium fluoride.
Audus (9,) claimed to have obtained complete inhibition of both 2,4-D and MCPA breakdown by 0.01% sodium azide in enriched soils. During the present investigation 0.1% azide was found to inhibit MCPA breakdown in the absence of soil

(Graph and Table 49,). 2,4-DCP breakdown appeared to be inhibited by 0.02% azide but no great reliance can be placed on the figure, for the rate in the presence of azide was not significantly lower than that observed in other, self-inhibited perfusers under similar conditions (Graphs and Tables 41, 69,). The breakdown of 4-CP was only slowed by 0.1% azide and it needed 1% to produce complete inhibition (Graph 68, Tables 68, 68c,). Sodium fluoride, which is generally similar to sodium azide in its action, was found to be relatively ineffective in inhibiting 4-CPA breakdown in the absence of soil. 0.1% and 0.5% were ineffective but 1% and 5% did prevent breakdown (Graph and Table 19,). Urethane, another respiration inhibitor, was completely inactive as a 2,4-D breakdown inhibitor at concentrations of 0.1% and 1.0%. It is possible that urethane was so labile in soil that it was impossible to maintain a concentration sufficiently high to exert toxic action on the adapted organisms.

The inability of these respiration inhibitors to prevent breakdown, except when present in relatively high concentration suggests that detoxication does not involve enzymic oxidation of the normal type. Inhibition at high concentrations was probably the result of general protoplasmic poisoning. It is probable that some oxidation reactions were involved for the frequent formation of pale coloured perfusates suggests the intermediate production of quinonoid or other highly conjugated compounds. Colouration was not observed in every perfuser and varied in intensity. It was always of the same amber-orange shade with a tendency to pink. Perfuser soil was

always obtained from the same source so the colouration cannot be attributed to soil extractives alone. It was never observed during perfusions of refractory compounds; it was not observed when a refractory compound was added to an active perfuser which had previously produced colour; it was observed most frequently and intensely with phenols; it reached a maximum intensity at times of most rapid breakdown and tended to disappear as the concentration of parent compound was reduced to zero.

It was observed by chance that all the perfusers in use fluoresced when exposed to ultra-violet radiation. The water, glass and soil-extract in use did not fluoresce. A solution of α-4-CPP likewise did not fluoresce but the perfusate drained from an active α-4-CPP perfuser (Graph and Table 64,) had a distinct pale blue fluorescence.

No attempt was made to follow up the colouration and fluorescence reactions.

II. The effect of various external factors on adaptation and retention of adaptation.

(a). Effect of concentration on adaptation.

The most commonly used concentration of phenoxy-acid in perfusate was 100 ppm. The labile compounds readily exhibited adaptation and breakdown phenomena at this concentration. 2,4-D was the only phenoxy-acid tried at 1,000 ppm. The pre-adaptation lag was only slightly lengthened but subsequent behaviour was erratic. Refilling at the same concentration often induced a second lag phase or a breakdown rate lower than expected. The greater persistence of 2,4-D at higher concentrations had previously been reported by Newman and Thomas (89,). 2,4-D, especially at high concentrations, has been shown by numerous workers (1, 19, 25, 37a, 57a, 58, 68, 77, 87, 96, 111, 117, 118, 138,) to be toxic to a variety of soil micro-organisms and it is to be expected that young active adapted cells, produced during later stages of the initial perfusion, would be very sensitive to the sudden change to a high 2,4-D concentration on refilling (cf. 142,). Apart from the toxic action of 2,4-D itself, a higher concentration of toxic breakdown products may have been operative. If such substances existed they must have been non-cumulative intermediates rather than end products for drainage of perfusers before recharging did not alter their subsequent behaviour (Tables 30d, 30e,).

Those phenoxy-acids which appeared to resist breakdown at

100 ppm. usually showed signs of activity at 10 ppm. even to the extent of giving normal adaptation and breakdown curves, (Graphs 18, 43, 43a, 44, Tables 18, 18a, 43, 43a, 44, 44a,). It is possible that breakdown of these compounds at 100 ppm. was prevented by their toxicity. On the other hand, breakdown was probably taking place at 100 as well as at 10 ppm. but that at the higher concentration the assay technique was not sensitive enough to detect the slow rate of change in concentration.

Only α-2,4-DCPP behaved in a manner the reverse of that described above. Adaptation took place at 100 ppm. but not at 10 ppm. No simple explanation can be offered.

Perfusion behaviour of 2,4-DCP had a marked dependence on initial perfusate concentration. There is little doubt that 2,4-DCP is very toxic to soil bacteria, particularly to unadapted ones (94, 129,). After an initial rapid absorbtion of about 20% of the 2,4-DCP a period of fairly slow breakdown followed in each case. Starting at 200 ppm. this breakdown was very slow and prolonged. Replenishment of the 2,4-DCP, with or without drainage, caused a repetition of the process and it was a long time before an apparently true adaptation occurred (60+ days). At 100 ppm. or less a similar sequence was followed but at a faster rate. As was pointed out earlier (p.145,) adaptation of soil bacteria to 2,4-DCP probably occurrs readily but the development of a 2,4-DCP resistant enriched population may be a more difficult process governed largely by the 2,4-DCP concentration.

(b). Effects, on adaptation, of perfusion in mixture with other, more resistant, compounds.

In a mixed perfusate, 2-CPA could not prevent adaptation to, and breakdown of, 4-CPA even at ten times the concentration of the labile component. It did have a significant effect on the breakdown rate and the pre-adaptation lag was slightly lengthened (Graph 22, Tables 22, 22a,). Inhibition was, apparently, not due to a reduction in numbers or damage to the 4-CPA adapted cells, for on draining and refilling with 4-CPA alone a normal breakdown rate was observed.

2,4-D reacted in exactly the same way to 2-CPA inhibition (Graph 34, Tables 34, 34a,).

MCPA at 10 or 100 times the concentration of 4-CPA in perfusate delayed adaptation to the later compound by over 10 days. At equal concentrations the lag was unaffected. In all three cases, however, (Graphs 54a, 54b, Tables 54, 54a, 54b,) the MCPA was decomposed, by the resultant 4-CPA enriched population, in much less time than the normal MCPA lag period. Subsequent batches of MCPA alone were broken down, though never with the rapidity of a true MCPA perfuser. The lag period for 2,4-D was also lengthened by perfusion in mixture with ten times its concentration of MCPA. Simultaneous addition of 4-CPA did not affect this result. When the MCPA and 2,4-D concentrations were equal, a normal 2,4-D lag was observed and all the MCPA was broken down by the thirtieth day (Graphs and Tables 55, 55a, 55b,).

As with 2-CPA, MCPA also inhibited breakdown of the more

labile component(s) in mixture with it.

The action of 2,CPA and MCPA in extending the pre-adaptation lag of 4-CPA and 2,4-D may have been due to a toxic action on the young dividing cells or even on the adaptation process itself. The build up of an enriched population would, in this way, be delayed and give the appearance of a lengthened lag phase. That the mature adapted cells were not permanently affected was shown by the ready breakdown of the labile compounds when they were subsequently added alone.

Reversable competitive absorbtion of resistant and labile compounds onto a limited amount of active surface, enzymic or otherwise, affords a more rational explanation of the inhibiting action of 2-CPA and MCPA on both pre-adaptation lag length and breakdown rate. The extent of inhibition would be proportional to the relative concentrations, and affinities for the active surface, of the labile and resistant compounds, if the available surface were the limiting factor. 2-CPA appeared to be unaffected by the breakdown of the labile compound but MCPA was not so inert. Once adaptation to the labile component had occurred there was a rapid fall in perfusate toxicity to a level lower than that required for the MCPA fraction. MCPA appears to compete for the breakdown sites but, unlike 2-CPA, it appears capable of being broken down itself at these sites. It may have a high affinity for the sites but, for steric or other reasons, is not such a good fit as the labile "template" compounds and hence its breakdown rate will be lower than normal.

Both 2-CPA/4-CPA and 2-CPA/2,4-D mixtures were more toxic in the cress test than could be expected from a simple summation of the component activities. Again, competitive absorbtion, this time at the sites of physiological activity in the cress test material, offers a likely explanation of the synergistic action.

Neither the synergistic effect nor the lag and breakdown modifying effects, outlined above, is likely to be of practical value owing to the amounts and cost of the required adjuncts. They do stress the necessity of using pure compounds in growth and other experiments.

2,4-DCP had a pronounced effect on 2,4-D breakdown whether the perfuser had been enriched to 2,4-D first or to 2,4-DCP. In a 2,4-D/2,4-DCP mixture the 2,4-D level usually remained little changed till the 2,4-DCP level had dropped almost to zero. The action of 2,4-DCP may have been, once more, due to competitive absorbtion though its toxic activity, possible role as an intermediate in 2,4-D breakdown or as a competitive metabolite cannot be discarded. The effect of 2,4-DCP in mixture with 2,4-D on the pre-adaptation lag phase of the later compound was not determined.

The effect of 2,4-DCP on 2,4-D adaptation and breakdown is of great practical importance for commercial samples of 2,4-D usually contain a fairly high proportion of 2,4-DCP and other phenols as well as other phenoxyacetic acids. The widely varying figures quoted for 2,4-D persistence may be due, in part, to the use of impure, commercial grade 2,4-D in field trials. Hansen (45,) had previously shown that the growth

regulating activity of MCPA could be considerably modified by the impurities normally accompanying it in commercial samples.

(c). Effects, on adaptation, of pre-treatment of the soil with other, related, compounds.

The concept of simultaneous adaptation has been much used in studies of bacterial metabolism (30, 31, 50 , 108, 109, 114, 115, 116, etc.,). The basic assumption is that micro-organisms adapted to the breakdown of a particular compound will not exhibit a lag phase when subsequently tested with intermediates in the breakdown chain. This assumption is not strictly valid unless the intermediate, at the concentration used, is no more toxic to the micro-organisms than the equilibrium concentration normally present during breakdown of the parent compound. The corollary of the assumption is also not necessarily true, that is , that adaptation to an intermediate will always result in a reduction of the lag phase of the primary compound. Such reduction would be dependent on the degree of resistance to breakdown offered by the intermediate compared with the preceeding stages of the complete breakdown.

Cross-adaptation is a similar phenomenon and may be said to exist when a reduction in lag phase for compound "A" is produced by pre-adaptation to compound "B" when it is clear that "A" cannot be an intermediate in the breakdown of "B". This is usually taken to imply that either, (i). there is a primary attack by a single enzyme system on

a structural feature common to both molecules or , (ii) there is a common intermediate and subsequent products linking the breakdown chains of the two compounds. There is, however, no reason to exclude the possibility that the enzyme system attacking one compound may be capable of rapid conversion to a system active against a similar compound, though the method of attack may be different in the two cases. The strain of organism adapted to one compound may be so conditioned that it can readily produce a further, independent, enzyme system active against another similar compound. Some of the 2,4-D / 2,4-DCP results suggest that such a set up may have been operative (Graphs 42c, 72, Tables 42b, 42c, 42d, 42e, 72,).

Pre-adaptation to 4-CPA completely obliterated the lag phase of subsequently added 2,4-D, α-4-CPP, and MCPA (Graphs 23, 24, 25, Tables 23, 23a, 23b, 23c, 24, 25,). It is obvious that not one of these compounds can be an intermediate in the 4-CPA breakdown chain. It is reasonable to assume that all four compounds are attacked by the enzyme system produced in response to 4-CPA treatment and must, therefore be regarded as examples of cross-adaptation. The result of 4-CPA pre-treatment on α-2,4-DCPP breakdown was inconclusive (Graph and Table 26,).

Simultaneous adaptation may be a correct interpretation of the observation that there was no pre-adaptation lag when 4-CP was added to a 4-CPA enriched perfuser for 4-CP is a possible intermediate in 4-CPA breakdown. In the light of the 2,4-D / 2,4-DCP results it is probably better to regard the bacterial population resulting

from 4-CPA adaptation as being capable of attacking 4-CP by a different enzyme system either immediately or after an imperceptible lag (Graph 27, Tables 27, 27a, 27b, 27c,).

As might be expected, cross-adaptation existed between 4-CPA and 4-IPA in a perfuser enriched to the later compound (Graph and Table 28,).

Curiously, there was no breakdown of POAA when added to a 2,4-D enriched perfuser (Graph and Table 32,) though it is a theoretically possible intermediate, is less likely to be toxic and might at least be expected to follow a similar breakdown path.

Pre-adaptation to 2,4-D did not promote breakdown of 3,4-D or 2,4,5-T (Graphs 36, 37, Tables 36, 37, 37a,) though all three have features in common including the 4-chloro substituent. On the other hand, cross-adaptation did exist between 2,4-D and MCPA which has the same common feature of 4-chloro substitution (Graph 38, Tables 38, 38a,). The possibility of different breakdown paths for 2,4-D and MCPA is supported by the isolation, by Jensen and Petersen (56,), of two very different bacterial species, one of which could could break down both 2,4-D and MCPA while the other could attack only 2,4-D.

4-CPA also has the 4-chloro group in common with 2,4-D and is a possible intermediate in the breakdown of the later compound. The absence of lag when it was added to a 2,4-D enriched perfuser may, therefore, be an indication of simultaneous adaptation.

The differing behaviour of the α-chlorophenoxypropionics when following 2,4-D enrichment is worthy of note. Lag-free breakdown of α-4-CPP suggests cross-adaptation. α-2,4-DCPP was apparently not attacked. It will be recalled that pre-adaptation to 4-CPA had the same effects. The implications of these results are discussed more fully at a later stage (see p. 188,).

At first sight, 2,4-DCP seems to be the most likely intermediate in 2,4-D breakdown, resulting from a hydrolysis of the ether linkage. The rapid, and apparently lag-free, breakdown of 2,4-DCP, at all tried concentrations, when added to 2,4-D enriched perfusers tends to support the theory and indicate simultaneous adaptation. Loss of activity on draining and refilling suggests that the theory is untrue and that other explanations should be sought. It is probable that 2,4-D treatment brings up a large population of young and active B.globiforme cells. These may have an independent enzyme system capable of dealing with low phenol concentrations and a ready breakdown of 2,4-DCP occurrs subsequent to the change over. Due to the toxicity of the higher phenol concentration, or of intermediates, there may be a rapid fall in activity of the population and in its numbers. Apart from general toxicity of the perfusate, the young cells in the perfuser might be expected to be less adaptable on first changing over to 2,4-DCP (cf. Zobell, 142,) and consequently the few remaining resistant cells may take some time before they build up into a new population truly active against, and resistant to, 2,4-DCP.

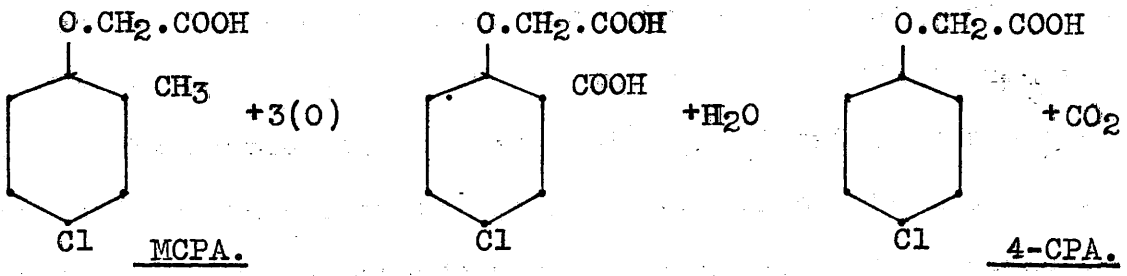
It is even possible that the new population capable of dealing with 2,4-DCP does not arise by division of 2,4-DCP resistant cells surviving from the original 2,4-D/2,4-DCP population but from a further strain of B.globiforme, adaptable to 2,4-DCP but not to 2,4-D, which had remained quiescent in the perfuser during 2,4-D enrichment. The co-existance of two such strains could account for the apparent simultaneous adaptation to 2,4-DCP in 2,4-D adapted populations and absence of simultaneous adaptation to 2,4-D in 2,4-DCP adapted populations. Relative speed of build up, and resistance to toxic action, of each strain could also be factors modifying the behaviour of both 2,4-D and 2,4-DCP enriched perfusers when treated with a mixture of the two compounds. For example, when a 2,4-D enriched perfuser was treated first with 2,4-DCP (which was broken down) and then with a 2,4-D/2,4-DCP mixture (Graph and Table 42,) there was evidence of inhibition of 2,4-D breakdown by the 2,4-DCP. Breakdown of 2,4-D accelerated when the 2,4-DCP had been reduced to a low level. The "two strain" explanation of this behaviour would be.....the 2,4-D / 2,4-DCP strain brought up by 2,4-D treatment gave way to the 2,4-DCP strain on treatment with this compound. This strain would still be dominant when the perfuser was switched to the mixture. It would only be when the 2,4-DCP level had been lowered sufficient, to become relatively non-toxic, that the original 2,4-D/2,4-DCP strain re-asserted itself and caused the 2,4-D to disappear. Further treatment with mixture could result in a gradual development of a balanced, mixed population capable of breaking down the two compounds

simultaneously. Similar behaviour was observed in perfusers enriched first to 2,4-DCP and later switched to 2,4-D / 2,4-DCP mixture. If 2,4-DCP is an intermediate in 2,4-D breakdown, it might be expected that, in a mixture, the 2,4-D would remain untouched till the 2,4-DCP had been lowered to the equilibrium level operating in a purely 2,4-D perfuser. In fact, 2,4-D breakdown did commence when the 2,4-DCP was still at a concentration higher than that of any phenol reacting compound detected in pure 2,4-D perfusers. It must be concluded that 2,4-DCP exerts its influence not by being an intermediate but by its toxicity and possibly by bringing up preferentially a second strain of organisms.

The effect of prior adaptation to 2,5-D on 2,4-D breakdown was inconclusive (Graph and Table 44,). The 2,4-D was broken down slowly over a period of about 30 days and may have been caused by independant adaptation of a small number of organisms surviving the 2,5-D treatment.

All the MCPA adapted perfusers were produced by the transferred adaptation process from a single primarily adapted perfuser and may be regarded as very similar, the adapted organisms in each possessing the same basic enzyme system. Perfusers adapted to MCPA showed an extraordinary fascility for dealing with related compounds, even when the only structural similarity was the side-chain. Most of these secondary compounds were used at the low level of 10 ppm. and it may be that what appeared to be simultaneous, or cross-adaptation, was often just the rapid re-adaptation of an existing hardy population to the new compound. The adapted

cells probably developed more slowly in response to the resistant MCPA and were not likely to suffer from the defects of young actively dividing cells which probably existed in perfusions of more labile compounds. The MCPA enzyme system may also have provided a starting point from which the organisms could overcome an otherwise insurmountable energy, or other, barrier in adapting to the even more resistant compounds. 2,CPA, 3-CPA, 4-CPA, 2,4-D, 2,4,5-T, 2,5-D, 3,4-D, 2,4-DM and 2,4-DCP were all broken down rapidly in one or more MCPA enriched perfusers. 4-CPA could, theoretically, be produced by oxidation of the methyl group of MCPA followed by decarboxylation.



Simultaneous adaptation would then be the correct term for the breakdown of 4-CPA by an MCPA enriched perfuser.
It is difficult to imagine the other reactions (involving 3-CPA,etc.,) as being examples of cross-adaptation unless the common mode of attack was on the ether linkage of the side-chains. As phenols never seemed to accumulate in these perfusers it is a necessary assumption that they also were broken down with, again, very little structural resemblance to account for a common method of breakdown.

In time there was a fall off in the rate of breakdown of 2,5-D and 2,4,5-T by MCPA enriched perfusers.

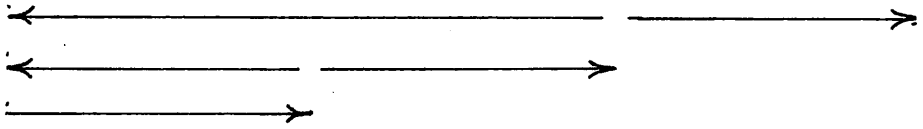
This could have been caused by accumulated toxic breakdown products. It is possible that the MCPA enzyme has only a limited affinity for these other substrates and consequently the rate of breakdown will tend to depend on the substrate concentration, the two decreasing together.

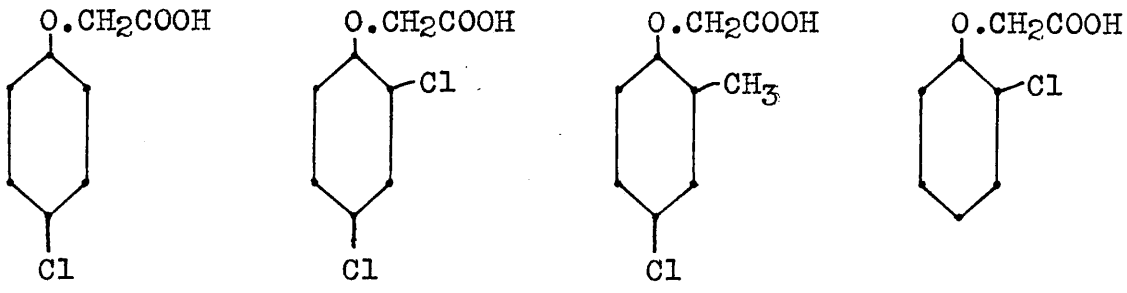
The inter-relationships of adaptation to 2-CPA, 4-CPA, 2,4-D and MCPA present a number of interesting points. MCPA enriched perfusers can break down 4-CPA and 2,4-D which are similarly substituted with chlorine in the 4-position, and also 2-CPA which is not similarly substituted. 2,4-D enriched perfusers can attack 4-CPA and MCPA which, again, resemble it in 4-Cl substitution but have no action on 2-CPA which has the same 2-chloro substitution. Pre-adaptation to 4-CPA promotes 2,4-D breakdown and possibly MCPA though the later observation is not very reliable. Apart from the acetoxy side chain common to all four compounds, the most obvious connection, in the breakdown relationships, is the 4-chloro substituent common to all three labile compounds. Against this, 4-CPA may not extend its influence to include MCPA and MCPA perfusers can attack 2-CPA which is without the common factor. If the compounds are arranged in order of decreasing lability (pre-adaptation lag phase is taken as a measure of lability) it will be found that there is a relationship between the compounds.

Lags.. 4-CPA $<$ 2,4-D $<$ MCPA $<$ 2-CPA.

4-CPA organisms can definitely attack the next most difficult compound, 2,4-D. 2,4-D organisms can attack the more labile 4-CPA and the next most resistant, MCPA. MCPA organisms are

active against both compounds below it on the scale as well as against 2-CPA which is above it. Direct adaptation to 2-CPA was never achieved so it is not possible to say whether such a population would be active against the other three compounds.





4-CPA 2,4-D MCPA 2-CPA

The mechanism underlying the relationships outlined above is probably one of activation energy levels. It is probable that primary adaptation to each compound excites the production of its own specific enzyme. These enzymes are probably very similar and attack the various compounds in the same way but only if the molecules fall within a limited activation energy range. It might be expected that these ranges will show some degree of overlapping and that enzymes responding to molecules of low activation energy will be able to attack those with overlapping higher or lower ranges. The expected order of molecular activation increases in the same order as the lability determined by the pre-adaptation lag phase and the above theory is, therefore, not without

foundation.

It is of interest to note that 2,4-DCP was broken down readily and repeatedly in an MCPA enriched perfuser without going into the dormant phase characteristic of 2,4-DCP treated 2,4-D perfusers. This suggests that the population developed in response to MCPA treatment is much more resistant to 2,4-DCP, or its breakdown products, than is a 2,4-D adapted population.

Prior enrichment to ∝-4-CPP did enable perfusers to deal readily and without lag with 4-CPA and 2,4-D. ∝-2,4-DCPP was only broken down at 100 ppm. when added to a 1,000 ppm. enriched ∝-4-CPP perfuser. Each case must be regarded as cross-adaptation for simultaneous adaptation is not possible. Unlike the reciprocal procedure, pre-adaptation to ∝-2,4-DCPP did permit lag-free breakdown of 2,4-D. This too is most likely a case of cross-adaptation for, though theoretically possible, it is most unlikely that 2,4-D is a normal intermediate in ∝-2,4-DCPP breakdown.

The complete cross-adaptability existing between the three compounds 4-CPA, 2,4-D and ∝-4-CPP with the virtual exclusion of ∝-2,4-DCPP cannot be interpreted simply as due to differences in activation energy for, though such differences probably exist, they are not likely to be large. Rather, the relative inertness of ∝-2,4-DCPP to the enzyme systems initiated by the other compounds must be due to a more complete blocking by the resistant and physiologically less active enantiomorph, or to steric effects

involving the 2-chloro nuclear substituent and the α-methyl group of the side chain.

In view of the cross-adaptation results involving 2-CPA, 4-CPA and 2,4-D, the effects of pre-adaptation to 2,4-DCP on adaptation to 2-CP and 4-CP were completely unexpected. Following on after 2,4-DCP, 2-CP was readily decomposed and subsequent additions of the same compound were broken down without signs of decreasing activity. When 4-CP was added to a 2,4-DCP enriched perfuser there was no evidence of simultaneous or cross-adaptation. Instead, a fairly normal adaptation to 4-CP seemed to occur. It will be seen that the behaviour of the phenols was the reverse of that of the corresponding phenoxyacetic acids. The implications will be discussed in a later section (p.181-2). Walker (129,) showed that 2-CP is more readily broken down, in soil, than 4-CP;an observation which is in agreement with those outlined above and which may offer an explanation of the cross-adaptation results.

The effects of previous 2,4-DCP adaptation on subsequent adaptation to 2,4-D were inconclusive but did not preclude the possibility of 2,4-DCP being an intermediate in 2,4-D breakdown. It is more likely, however, that given suitable conditions the population built up in response to 2,4-DCP treatment can quickly evolve an enzyme system capable of dealing with 2,4-D.

(d). Effects of intermediate adaptation to, or perfusion with, a second compound on retention of a primary adaptation.

In several instances, perfusers were returned to the initial enriching compound after testing for cross or simultaneous adaptation with second compounds.

4-CPA adaptation was not broken by intermediate perfusion with 4-CP over a long period. The 4-CPA organisms must have persisted through the 4-CP treatment, or only one species was involved in both reactions. The later is possible for 4-CPA pre-adaptation had allowed active, lag-free 4-CP breakdown.

2,4-D adaptation was not broken by intermediate perfusion with 2,4-DCP or 2,4-DCP / 2,4-D mixtures.

MCPA adaptation was unaffected by intermediate 4-CPA adaptation. It was also retained through 2,4-DCP adaptation though the breakdown rate was reduced on returning again to MCPA. This may have been caused by the general toxicity of 2,4-DCP.

α-4-CPP adaptation was not broken by 4-CPA.

2,4-DCP adaptation was unaffected by intermediate adaptation to either 4-CP or 2,4-D.

In no experiment did perfusion with a second compound cause loss of adaptation to a primary treatment. The MCPA / 2,4-DCP and some of the 2,4-D / 2,4-DCP results suggest some loss of activity but this was probably the result of 2,4-DCP toxicity rather than a more specific physiological action. In each case, too, the non-effective compounds had been found to exhibit cross or simultaneous

adaptation phenomena with the primary adaptors. It seems highly likely that the same population, with or without minor modifications, was acting on each pair of compounds.

Intermediate perfusion with a resistant compound such as 2-CPA might have had some effect on adaptation retention though the results of 2-CPA / 4-CPA and 2-CPA / 2,4-D mixture perfusions suggest that temporary slowing down of the previous rate of breakdown is all that might be expected to occur.

(e). Effect of perfusion with water only, on retention of 2,4-D adaptation.

Perfusers were shown to retain their ability to break down 2,4-D for at least 60 days when only water was being circulated. It is unlikely that the adapted population remained completely inactive during this time. Cell division and metabolism of normal soil constituents must have continued during the water perfusion without producing a marked loss of the 2,4-D breakdown system. As the responsible organisms seem to be so capable of reverting to their normal soil substrates it is even more enigmatical that they should ever adapt and proliferate, in the soil, on such unusual substrates as the phenoxyacetates. It would be of value and interest to know the normal soil substrates for these organisms.

(f). Retention of 2,4-D adaptation in stored soil.

Anderson and Baker (3,) found little residual activity against 2,4-D in pre-treated soil which had been stored between seasons at 34°F. Newman and Thomas (89,), Newman, Thomas and Walker (90,) and Newman and Norman (91,) have all reported persistence of 2,4-D adaptation in soil, even up to a year after treatment. Persistence was mainly studied under field conditions.

The tenacity with which 2,4-D adaptation was retained, once it had become firmly established in an enriched population, was demonstrated clearly by the results of experiments using stored soil.

A large batch of enriched soil was produced in an aluminium perfuser column (Table 31,). The column was stored, with the damp soil under semi-anaerobic conditions, for one year. The soil was then taken out, air-dried, sieved and used to prepare perfusers in the usual way. In these perfusers, little lag was shown in the breakdown of 2,4-D at 100 ppm. and it was complete in less than the normal lag time for the compound (Graphs 39, 40, Tables 39, 39a, 40, 40a,). It is again surprising that the 2,4-D enzyme system should have been retained intact as part of the enzymic complement of the organisms for they must have been exercising other metabolic powers during the year of storage. The implications of these observations may be serious for the use of selective herbicides in practice. If anti-herbicidal activity is retained under field conditions it means that subsequent applications of herbicide will be much less effective than the initial one. Newman and Thomas (89,)

and Newman and Norman (91,) have already drawn attention to this possibility.

III. Resistance to attack and possible course of breakdown in relation to molecular structure.

(a). Breakdown via corresponding phenols.

Synerholm and Zimmerman (120,) showed that for the series of ω-(2,4-dichloro phenoxy-)aliphatic acids, only those with an even number of carbon atoms in the side chain were physiologically active. Fawcett, Ingram and Wain (33,) showed that for the similar homologous series of ω-phenoxyaliphatic acids a periodicity also existed in their behaviour in flax plants. Those acids which contained an even number of carbon atoms in the side chain were not obviously affected but those containing an odd number were broken down to give phenol. They believed that the odd numbered side chains were degraded by repeated β-oxidation till only phenol remained. The even numbered side chains were either unaffected or β-oxidation necessarily ceased when the side chain had been shortened to that of phenoxyacetic acid. Under the experimental conditions, no phenol was produced from phenoxyacetic acid. Synerholm and Zimmerman also postulated β-oxidation of the side chain to explain their findings.

Consequently, if the results of Synerholm and Zimmerman can be combined with those of Fawcett et al, it follows that 2,4-D or any other nuclear substituted phenoxyacetic acid cannot be

expected to give rise to the corresponding phenol when degraded in plants. The assumption has to be made that all plants would act in the same way as flax if they did act on the phenoxyacetic acids. There is no obvious reason why a different breakdown mechanism should operate in bacterial attack on the phenoxyacetic acids and it therefore appears likely that the oxyacetic side chain will remain intact and that a phenol will not be produced by hydrolytic or other attack on the ether linkage.

The fascility with which other compounds were decomposed by MCPA enriched perfusers would suggest that the side chain is the most likely point of primary attack on the molecule for the side chain was often the only feature common to the two compounds in question. The widely differing primary lag phases of these compounds, and the fact that in some cases adaptation did not seem to occur at all, tends to contradict the above reasoning for nuclear substitution cannot be expected to have such a marked influence on the attack on a common side chain structure. The similarity in lag phase of 4-CPA and ∝-4-CPP lends support to this later view, for in these compounds the nuclear substitution is identical and it is the side chain structures which differ. Similarly, 4-CPA and 4-IPA have very different lag phases though the side chain and substitution in the 4-position is common to both molecules. Only the character of the nuclear substituent is differemt.

Phenoxyacetic acid was found to resist attack much more than 4-CPA or 2,4-D. This is not to be expected if a phenol is the first major breakdown product in the degradation chain.

4-CPA and 2,4-D would give 4-CP and 2,4-DCP respectively, both of which were found to offer some resistance to breakdown and to exhibit toxic properties. Other workers have found the chlorinated phenols to be more toxic and/or resistant to attack (22, 79, 94, 129,) than is phenol (expected from POAA). It is known that, at the maximum concentration at which it could have occurred in a POAA perfuser, phenol is quite labile and relatively non-toxic in soil.

Phenol reacting compounds (Folin and Ciocaleu Method, p.46,) were never found in quantity at any time during herbicide perfusions. This is not absolute proof that phenols are not breakdown intermediates for intermediates in any system need not accumulate to any extent. In addition, the phenols 4-CP and 2,4-DCP seemed to be fairly labile at the lower range of concentrations. There was some evidence of an increase in phenol reaction during the fluoride inhibition of 4-CPA breakdown (Graph and Table 35,). The increase in phenol reaction was probably not significant and may have been an artefact resulting from interference in the test by high fluoride concentration or by organic debris from the perfuser. 4-CP added to soil tended to fall in concentration to an equilibrium point of very slow disappearance and there seems to be no reason why, if it is formed as an intermediate in 4-CPA breakdown, it should not rise to the same level so long as 4-CPA is still present. Walker (129,) found 4-CP to disappear only slowly from soil and that further 4-CP treatment had no effect on the rate of disappearance. Contrary to the present researches he found that 4-CP pre-treatment completely

prevented 4-CPA breakdown. He showed 2-CP disappearance to be a biological process and that this phenol was much less persistent than 4-CP or 2,4-DCP. 2-CP was not tested, during the present work, for direct adaptation but the results of simultaneous adaptation tests with 2-CP and 4-CP after 2,4-DCP adaptation do support Walker's findings. The reverse order of lability of the corresponding phenoxyacetic acids 2-CPA and 4-CPA, in which 4-CPA had a short lag and 2-CPA did not seem to break down at all, again suggests that the phenols cannot be intermediates in the breakdown chains.

Most of the 2,4-D / 2,4-DCP and 2,4-DCP / 2,4-D cross-perfusion results are ambiguous, neither supporting nor condemning the intermediate phenol hypothesis. It is probably better to regard them as showing that both compounds can be attacked by the same enriched population with, or without further modification. Some of the 2,4-D / 2,4-DCP mixture results are likewise ambiguous while the others are best interpreted on the assumption that the two compounds are broken down independently by the one population.

Unpublished work by W.C.Evans suggests that 4-CPA is broken down via the phenol. Hydroxylation occurrs first to give 4-chloro,2-hydroxyphenoxyacetic acid which is then split at the ether linkage to give 4-chlorcatechol. This gives rise to chlorinated fatty acids after ring fission. He thinks that 2,4-D does not follow a similar path.

The general conclusion to be drawn from all the above evidence is one of "not proven", with slight bias in

favour of the opinion that phenols do not usually figure in the breakdown schemes of the phenoxyacetic acids.
If it is assumed that the ether linkage is not the primary point of attack on the molecule, it remains to be decided at which point attack is most likely to take place. A study of the resistance to attack, as measured by the pre-adaptation lag, in relation to the molecular structure of several halogenated phenoxy acids provides a valuable clue.

(b). Relationship between structure and resistance to attack.

It was found that the halogenated phenoxyacetic acids tested were divisible into two fairly distinct groups:
(i). those which were broken down with ease, after a relatively short lag phase, when perfused at 100 ppm.
(ii). those which were apparently not attacked even after long perfusion at this concentration.
Group(i) consisted of 4-CPA, 4-IPA, 2,4-D, 3,4-D and also the unsubstituted POAA and propionic acid derivatives α-4-CPP and α-2,4-DCPP.
Group(ii) contained 2-CPA, 3-CPA, 2,5-D and 2,4,5-T.
MCPA resembled Group(i) compounds though it had the much longer lag of about 70 days. MCPA cannot really be compared with the straight halogenated acids for the presence of an oxidisable methyl group substituted in the nucleus offers another point of attack on the molecule not available in the straight halogenated acids. A methyl substituent also differs in other ways from a halogen and the erratic behaviour of the doubly substituted 2,4-DM may only have been an extension of

the trend beginning with MCPA.

Of the Group(i) compounds it will be seen that only one, namely POAA, is not substituted in the 4-position while of the Group(ii) compounds only one, ie., 2,4,5-T is so substituted.

Nuclear substitution can modify attack on a molecule in two principal ways.

(a). by activating, or de-activating, the nucleus in certain positions by altering its mean electronic configuration, ie. resonance effects.

(b). the relative bulk and position of the substituents can hinder attack at certain points, eg. on adjacent carbon atoms, or further distant by restricting rotation of side chains, etc., ie. steric effects.

The side chain -O-CH(R)-COOH, where R is H or CH_3, is common to all the compounds in both lag-phase groups and can be expected to have weak ortho and para directing properties for cationoidal agents. The halogens are fairly powerful ortho/para directors though they have a general de-activating effect on the molecule. The methyl group is almost equal to the halogens in its ortho/para directing abilities but has little or no de-activating effect. A point which may be of great significance is the fact that oxidation can convert the ortho/para directing methyl group into the medium strength meta directing carboxyl group. Methyl groups have been shown to be capable of oxidation in other compounds (14,). If the methyl groups of MCPA and 2,4-DM behave in the same way, the somewhat

anomalous behaviour of these compounds may find an explanation.

By analogy, from evidence presented in a later section on the relationship of herbicidal activity to molecular structure and from the observations of other workers (94a, 110, 112,), it is reasonable to accept the hypothesis that an α-H atom and the carboxyl group of the side chain together with some point on the nucleus are involved in the enzymic breakdown of phenoxy-acids, as well as in their physiological activity. It is this third attachment point, on the nucleus, which is no doubt controlled by the nuclear substituents.

Group(i) compounds. The parent compound POAA might be expected to be weakly activated, towards cationoidal agents, in the ortho and para positions (only the oxyacetate radical is acting on the nucleus) ie. at the 2, 4 and 6 carbon atoms. There should be no activation of the 3 and 5 positions. With 4-CPA both substituents are ortho/para directing but are antagonistic as they are para to each other. The nett result is most probably activation of the 3 and 5 positions by the more powerful halogen atom. The two positions will be equivalent owing to the symmetry of the molecule and lack of steric hindrance. Consideration of all the results suggests that relative activation of the 3 and/or the 5 position(s), with attachment of the enzyme to one or other of them (the third point), is the controlling factor in the breakdown of these molecules. The marked lability of 4-CPA is consistent with the theory of equally activated and unhindered 3 and 5 positions in the molecule. The 3 and 5 positions of 4-IPA

will again be equivalent and activated though the iodine atom is slightly less activating and may, because of its bulk, have a hindering effect on 3 or 5 attachment. It was easily broken down in 4-CPA enriched perfusers but, as might be expected from the above theoretical considerations, had a lag greater (1.5 to 2 times) than that of 4-CPA.

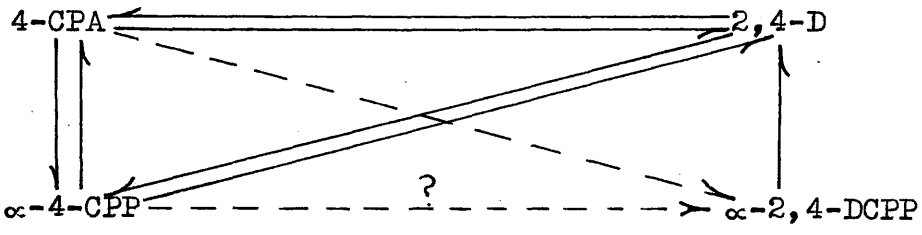
In 2,4-D the ortho/para directing powers of the two chlorine atoms can be expected to be additive but the 4-ortho,2-para effect at the 5-position will probably be greater than the 4-ortho,2-ortho effect at position 3. The slight inactivating effect of the oxyacetate radical on these two positions and the possibility of steric hindrance in the 3-position combine with the other factor to make the position 5 the most likely point of attack. The slightly greater resistance of 2,4-D to attack may be due to there being only one favoured point of attack wheras there are two equal ones in 4-CPA.

In 3,4-D, attachment at the 3-position is most probably blocked by the substitution. It is difficult to predict whether the 5 or the 6-position will be most activated by the resultant action of the three substituents. It is possible that both are activated to some extent, activation of the 5-position being less than in 4-CPA and 2,4-D and consequently reflected in a much longer lag phase than that of either of these compounds.

α-4-CPP and 4-CPA differ only in the side chain and the difference cannot be expected to have much effect on the status of the nucleus. The almost identical perfusion behaviour of the two compounds confirms this view. The results of α-4-CPP

breakdown experiments suggest that both enantiomorphs are broken down with little mutual interference.

Perfusion results for ∝-2,4-DCPP suggest that only one of the isomers is degraded and that it is the more physiologically active of the two. It has been shown (110,) that the weakly active (-) isomer can antagonise the more powerful physiological activity of the (+) isomer. It seems probable that a similar inhibition operates during the breakdown of ∝-2,4-DCPP for, though there is a possibility of interference between the methyl group of the side chain and the 2-chlorine and so preventing correct orientation of enzyme and substrate, the molecule is otherwise so like 2,4-D that there appears to be no other explanation of the marked difference in lag phase of 40 to 60 days compared with 10 to 14 for 2,4-D. On the other hand, such enantiomorph antagonism was not observed with ∝-4-CPP. Steric effects involving the ∝-CH_3 group of the side chain and the 2-chloro group on the nucleus of ∝-2,4-DCPP and the absence of such effects in ∝-4-CPP may form a basis for explanation of the relative inertness of ∝-2,4-DCPP and the way it apparently stands apart from the completely inter-adaptable group 4-CPA, 2,4-D and ∝-4-CPP.



Pre-adaptation to any one of the above compounds permits breakdown of any other joined to it by an arrow. The broken

arrows indicate doubtful activity.

Group(ii) compounds can now be examined to see if an explanation of their inertness can be found along the lines followed with the Group(i) active compounds.

The two substituents in 2-CPA are both ortho / para directing and by mutual interference probably result in lack of activation or even in de-activation of the molecule. The 2-Cl substituent may also have a hindering effect on enzymic attachment at the 3-position so the molecule will be less susceptible to attack, particularly if it is, at the same time, inactivated at the 5-position.

In 3-CPA there should be a combined activating effect of the two substituents leading to activation of the 2, 4 and 6 positions. The 5-position will be inactivated and, as the 3-position is blocked by substitution, the unreactiveness of the compound is to be expected. It was found to be stable at 100 ppm. and only slowly affected at 10 ppm.

The most likely position to be activated in 2,5-D is the 6-carbon atom. The 5-position is blocked by substitution and the 3-position is hindered by the 2-chloro group. As might be expected the compound was resistant to attack.In soil it appeared to be broken down eventually and it is possible that the attack was made at the slightly activated 3 or 6 positions, or more likely at the less obstructed, mildly activated, 4-position. This later hypothesis finds support in the observation that substitution of a further Cl atom in position 4,to give 2,4,5-T, produces

an even more stable molecule. As well as its blocking effect, the 4-Cl atom would tend to lower further the slight activation of the 6-position. Newman et al (90,) attempted to explain the stability of 2,4,5-T by concluding that it was a poorer source of energy, for the bacteria, than 2,4-D. This naive conclusion does not indicate why 2,4-D should be broken down fairly easily when it is probably a poorer source of energy than POAA or the normal soil substrates of the organisms.

If then it can be assumed that the enzyme involved in breaking down the phenoxyacids needs to become attached at three points on the molecule, two on the side-chain and one on the nucleus, it follows from the above discussion that:

(A). the 3 and 5 carbon atoms of the nucleus are the most likely points of attack.

(B). substitution in the 3 and 5 positions will hinder attack.

(C). breakdown will be fascilitated by substitution of a powerful ortho/para directing group in position 4.

(D). substitution in the 2- and 6- positions may affect activation of the molecule as a whole but is more likely to inhibit breakdown by steric hindrance of enzyme attachment to the 3- or 5- position.

(E). it is probable that breakdown would be promoted by a meta directing substituent in the 3- or 5- position, or better by a meta director at position 3- and a strong para director in position 4-.

IV. Effect of molecular structure on physiological activity as determined by the cress-test.

The work of Wain and others (94a, 110, 112,) seems to indicate that physiologically active phenoxy acids become attached to their substrates (enzymes?) at the α-H and carboxyl group of the side chain and at a point on the nucleus. Muir and Hansch (85, 85a,) and McRae and Bonner(78,) support the theory that it is a position ortho to the side chain which is the nuclear focal point, they found 2,6-di and 2,4,6-trichlorophenoxyacetic acids to be inactive (85a,) and weakly active and active as anti-auxins (78,) respectively. Other workers have cast serious doubts on the theory (124, 127,). Phenoxyacetic acids substituted in both ortho positions have been shown to be active, eg. Wain showed 2,4-dichloro,6-fluorophenoxyacetic acid to be an active growth substance while Zimmerman (139,) found the corresponding 2,4,6-trichlorophenoxyacetic acid to be inactive in promoting cell elongation. From a study of all the mono- di- and tri- chlorophenoxyacetic acids, Leaper and Bishop (63,) concluded that two unsubstituted positions,para to each other, such as 2 and 5 or 3 and 6 were necessary in order to confer physiological activity on the molecule. Further, they concluded that a quinonoid structure resulting from the oxidation of such compounds might exert growth regulating action by participating in an oxidase system. It will be recalled that quinonoid breakdown intermediates were suggested as an explanation of the colour formation in active perfusers (p.147, 159,). Leaper and Bishop also

postulated further active breakdown products related to maleic acid.

Attempts to correlate the vast number of results obtained by many workers, on the relationship of molecular structure to physiological activity, have been complicated by the many different tests for activity which have been used. Also, it has not always been appreciated that (a). the different types of growth regulators could produce similar overall effects by acting on different inter-connected systems, or different parts of the same system, and therefore need not necessarily have structural features in common. (b). the observed activity might not be a function of the parent compound but of breakdown, or other modified, products. Comparison of the physiological activities of some substituted phenoxy acids has therefore been made with these limitations in mind. Where necessary, comparison with other results has only been made when they were obtained with similar test procedures.

Relative Toxicity determined by the cress-test was taken as a measure of physiological activity and the compounds were arranged in order of decreasing Relative Toxicity. (Results tabulated on page 193a).

The cress-test depends to a large extent on the ability of the compounds to control cell elongation in the roots. Zimmerman (139, 140,),using cell elongation as a criterion of activity, placed 2-CPA, 4-CPA, 2,4-D, and 2,4,5-T in virtually the same order as found by the cress-test.

The Relative Toxicity table falls into two clearly distinguishable sections which, with one or two

minor exceptions, contain the same compounds as the two sections based on the pre-adaptation lag phases. Though no phenoxy acids were tried in which both ortho positions were substituted and none lacking the two free para positions specified by Leaper and Bishop, the evidence suggests that activity is associated with other features of the molecule. Activity seems to be associated primarily with the powerful ortho/para directing chlorine atom substituted at the 4-position. (Burström, 18, found para-chloro substitution in ∝-phenoxyisobutyric acids to be the most effective promoter of anti-auxin activity).

4-CPA is very much more active than POAA by reason of this 4-chloro substitution. 4-IPA which came into the same lability group as 4-CPA (previous section, p.184,) is here found to be in the opposite activity group, having the low Relative Toxicity of 5. The longer lag of 4-IPA compared with 4-CPA was attributed to the slightly weaker directing power of the iodine atom and steric hindrance due to it having a bigger effective diameter than the chlorine atom. There seems reason why the same factors should not be responsible for the more effective lowering of the physiological activity of 4-IPA.

Though very active, ∝-4-CPP is only about half as effective as 4-CPA. Side chain substitution may have an effect (steric) but it is more likely that the overall activity of the racemic mixture is lower due to antagonism of the more active (+) isomer by the weakly active (-) isomer,

Relative Toxicity Classification.

Section I. High values.

Compound.	Relative Toxicity.
MCPA	140.
4-CPA	100.
2,4-D	100.
∝-2,4-DCPP	67.
∝-4-CPP	50.
2,4-DM	18.
2,4,5-T	17.

Section II. Low values.

Compound.	Relative Toxicity.
2,5-D	8.5
3,4-D	5.5
4-IPA	5
3-CPA	4
2-CPA	4
POAA	0.2

(cf. 1a, 110, 112,). As the racemic mixture contains only 50% of the active isomer it cannot be expected to have a Relative Toxicity of more than about 50 even if the more active isomer is of the same order of activity as 4-CPA.

Substitution of a further chlorine atom in the 2-position of 4-CPA results in a compound, 2,4-D, with the same Relative Toxicity and, as was shown previously, with only a slightly longer lag.

Substitution of the weaker ortho/para directing methyl group in the 2-position of 4-CPA gives MCPA of much higher Relative Toxicity. MCPA activity may be due to the intact parent molecule or to breakdown products such as the aromatic acid resulting from oxidation of the methyl group. This substance could probably give 4-CPA by decarboxylation and it, or its breakdown products, be responsible for the activity. The Relative Toxicity of MCPA is almost certainly significantly different from that of 4-CPA and the prolonged pre-adaptation lag of MCPA also detracts from the hypothesis. The lag for MCPA is about 60 days longer than that for 4-CPA which means that the cress seedlings would need to accomplish in about 24 hrs. that which takes the soil micro-flora 60 days ie. breakdown of MCPA to 4-CPA. The theory cannot, however, be completely discarded without further evidence for a somewhat similar situation is found on comparing 2,4-DM with its related compounds. Thomson et al (125,) found 2-chloro, 4-methylphenoxyacetic acid to be a much weaker growth regulator than 4-chloro,2-methylphenoxyacetic acid (MCPA) with 2,4-DM occupying an intermediate position. Substitution

of the second methyl group reduced the Relative Toxicity from 140 for MCPA to 18 for 2,4-DM. Though the ortho/para directing powers of the methyl group are slightly lower than those of the halogens, this seems insufficient to account for the great drop in Relative Toxicity. The greater hindering effects of the methyl group also cannot account fully for the loss of activity. Pre-adaptation behaviour of 2,4-DM was erratic but it did seem to be a little more labile than MCPA and possibly little different from POAA. 2,4-DM could give rise to POAA by successive oxidation and decarboxylation of both methyl groups but it should be noted that the Relative Toxicity of 2,4-DM is higher than that of POAA. If cress seeds have the power to bring about the conversion suggested above, it offers a more reasonable explanation of the small effect of di-methyl substitution on the physiological activity of POAA.

The lower activity of α-2,4-DCPP (about 50%) compared with 2,4-D probably finds, again, a reasonable explanation in one or both of the ways suggested for α-4-CPP relative to 4-CPA. Either (a). physiological activity of the more active (+) enantiomorph is antagonised by competition with the weaker (-) isomer for the same substrate, or (b). the racemic mixture has only about half the Relative Toxicity of the more active isomer as it only constitutes 50% of the mixture. If antagonism is neglected, it follows that the more active isomer must be about as active as 2,4-D, molecule for molecule.

The low Relative Toxicity of 3,4-D is somewhat

anomalous as it was fairly readily broken down in soil. It can only be assumed that the de-activating effect of the 3-chloro substituent operates more effectively against plant growth enzymes than it does against catabolic bacterial enzymes. In both 3,4-D (otherwise 4,5-D) and 2,5-D the ortho/para directing chlorine atoms are ortho and para to each other,respectively,and in consequence their activating effects are antagonistic, resulting in low toxicity. In 2,4-D there is a reinforced action because the chlorine substituents are meta to each other. In 2,4,5-T the 2- and 4- substituents tend to activate the 3- and 5- positions, mainly the later. The 5-chloro substituent blocks this position as well as tending to counter the activating effects of the other two. The 3- position is blocked by the steric hindrance of the 2-chloro substituent. The overall effect is to produce a molecule with relatively low toxicity and high resistance to breakdown.

Thimann (124,) showed 3,5-D to be inactive as a growth substance; it has no activating 4- substituent and both 3- and 5- positions are blocked by substitution. Although posessing the requisite 4-chloro activator, 3,4,5-T would probably prove very inactive and very resistant to breakdown.

2-CPA and 3-CPA also lack the activating 4- substituent and were found to have very low Relative Toxicities as well as being resistant to attack in soil.

The evidence outlined above, though obtained with a relatively small range of compounds, suggests that the bacterial enzyme bringing about decomposition of the herbicide molecule reacts with certain key points on the activated molecule in a manner very similar to that in which some enzyme from plant growth regulating systems also becomes involved. The agreement between the two systems of classifying the compounds is too close to admit coincidence. One of the attachment points (probably one of three) is probably associated with the nuclear atom(s) meta to the side chain. If these meta positions are sterically hindered, or blocked by substitution, three point attachment cannot occur and an inactive, stable compound results. Compounds of this type may be more readily absorbed at what Veldstra (127,) calls "loss sites". Inactivity and resistance to breakdown could also be accounted for in this way. It would also offer a reasonable explanation of the apparently synergistic action of 2-CPA in mixture with 4-CPA or 2,4-D.

If the three point attachment theory (two on the side chain and one at a meta nuclear position) is accepted it remains to be decided whether the intact herbicide molecule alone acts on the growth regulating system because of its direct attachment to an enzyme or whether the enzyme further resembles its bacterial counterpart by bringing about disruption of the herbicide molecule. The primary,or subsequent, breakdown products may be the actual active substances or, alternatively, have greater activity than the parent compounds. Leaper and Bishop (63,) postulated toxic

breakdown products, from the chlorophenoxyacetic acids, related to the maleic acids. Many authors (7, 23, 62, 89, 90, 97, 123,) have inferred the formation, under both field and laboratory conditions, of active (toxic or stimulatory) breakdown products. With many biologically active compeunds stimulation and inhibition are a function of concentration. During the present work, some evidence has been produced suggesting breakdown without loss of activity, ie. to a more toxic breakdown product (see p.87,) and there have been several cases of increasing perfusate toxicity such as might be expected if breakdown products more toxic than the parent compounds were being produced (Graphs and Tables 20, 43a, 45, 46, 63, Table 59a,).

If attachment of the enzyme to the 3- or 5-position of the nucleus is followed by fission of the ring in that region, a complex, substituted, di-aldehyde would probably be formed, oxidisable in stages to a substituted adipic acid (Newman and Thomas, 89, obtained evidence of molecular oxidation without loss of activity). One or other of the chlorine atoms adjacent to the split might be liberated as inorganic chloride as was suggested by Hansch et al (44a,).

Biotin contains an adipic acid residue as a side chain to one of its two heterocyclic rings. Incorporation of a substituted adipic acid into such a molecule, giving a substituted biotin, is not beyond the bounds of possibility. Pimelic acid, the next higher homologue of adipic acid, is known to be capable of replacing biotin as a growth factor for

Corynebacterium diptheriae and the beetle Tribolium confusum. It also greatly increases the biosynthesis of biotin by Aspergillus niger when added to culture media.

A complex, substituted biotin would almost certainly not have the same physiological activity as biotin itself for even minor substitutions seriously lower its potency. It could be an antagonist for biotin in normal metabolism and, as biotin is known to be required in minute concentrations only, sufficient anti-biotin could be produced by breakdown of a fraction of the lowest herbicide concentrations used. Evidence lending some support to this theory has been obtained by workers studying the breakdown of radio-active 2,4-D in plants. Weintraub et al (135,) found that much of the absorbed 2,4-D was quickly broken down, the major breakdown product being a relatively volatile, or unstable, acidic substance. Holley (53, 54,) found that it was converted in 6 hrs. (the time required for the first visible effects of 2,4-D to appear) into a water soluble product, acidic or hydrolyseable to an acid.

In one experiment biotin was added to a perfuser and appeared to temporarily increase the rate of 2,4-D breakdown. This could be due to antagonism of the added biotin towards the substituted biotin breakdown product in the cress-test. An apparently lower herbicide concentration would be recorded. The herbicide concentration appeared to rise again later, which is to be expected if the added real biotin was broken down faster than the pseudo-biotin breakdown derivative.

If the above hypotheses are correct, the differential response of species to treatment with any"active" phenoxyacetic (or phenoxypropionic) acid may be interpreted in several ways.

(a). resistant species may not have the enzyme system which breaks down the parent compound to the toxic intermediate or other derivative. Alternatively, both resistant and sensitive species may possess the enzyme system but the reaction is prevented in the resistant species by a specific inhibitor. Goldacre (37b,) found an analogous situation in the oxidation of β-indolylacetic acid by an enzyme in the presence of hydrogen peroxide. Boiled onion bulb juice inhibited the reaction but boiled pea-epicotyl juice did not.

(b). resistant species may be able to eliminate the toxic compound quickly, whether it be the original phenoxy-acid or a derivative. Again, the presence or absence of inhibitors may be the controlling factor.

(c). resistant species may not be able to build up the phenoxy-acid derivative into an anti-metabolite or anti-growth-factor whereas the sensitive species are able to do so.

(d). resistant species may have such an excess of the normal metabolite or growth-factor that the concentration of "pseudo biotin" type of compound never becomes high enough to interfere.

(e). the metabolic pathway involving the growth regulator (parent or derivative compound) may be of only minor importance in the overall functioning of the resistant species.

Fang and Butts (32,) isolated two major

derivatives of radio-active 2,4-D from treated wheat and corn plants (resistant species) but only one of these was found in extracts from treated beans (sensitive species). It may be that the compound found in beans is toxic and cannot be further degraded by those plants. In corn and wheat it may be broken down to the second (non-toxic?) compound at least. This is a type (b). interpretation.

The growth regulating activity of substituted benzoic acids and, indeed, of all growth regulators which incorporate a six carbon ring, may be capable of interpretation along similar lines. Substituted adipic aldehydes may thus become the common link between what is otherwise a very heterogeneous collection of metabolicaly active compounds.

Supporting evidence for the anti-biotin theory may be derived from the results obtained by other authors in other types of experiment. Dubos (29,) found that the presence of peptone or tryptophane in the medium would partly or completely remove growth inhibition, of some bacteria, by synthetic hormones,including 2,4-D. Conversely, during the present series of experiments, some evidence was found that 2,4-D could inhibit utilisation of peptone by B.globiforme (see pp. 62, 71,). Biotin has been shown to play a part in amino-acid oxidation (68a, 123a,), in particular with the oxidation of tryptophane to kynurenine in the chain of reactions leading to the formation of nicotinic acid from its precursor indole (103,). Biotin was thus shown to be directly connected with nicotinic acid, itself a growth factor, and possibly β-indolylacetic acid via indole or its precursor.

These workers found that addition of the biotin anti-metabolite γ-(3,4-urylenocyclohexyl-)butyric acid , prevented the normal, biotin catalysed, reaction causing tryptophane to accumulate. Further addition of an excess of biotin allowed the normal reaction to procede.

It seems reasonable to suppose that if the breakdown products of 2,4-D, etc., can be built up by the plant into biotin anti-metabolites (of the 3,4-UOCHBA or "pseudo-biotin" types) they would similarly disrupt amino-acid oxidation and nicotinic acid production, with marked effects on the plant's growth and metabolism.

REFERENCES.

1a. Åberg, B., Physiol. Plantarum, 5, 305 (1952).

1. Akamine, E.K., "Plant growth regulators as selective herbicides". Hawii Agric. Exptl. Stn. Circ., 26, 1-43 (1948).

2. Akamine, E.K., "Persistence of 2,4-D toxicity in Hawiian soils". Bot. Gaz., 112(3), 312-319 (1951).

3. Anderson, G.R., and Baker, G.O., "Some effects of 2,4-D in representative Idaho soils". Agron. Jour., 42(9), 456-458 (1950).

4. Arakeri, H.R., and Dunham, R.S., "Environmental factors relating to the pre-emergence treatment of corn with 2,4-D and soybeans with TCA." Minnesota Agric. Exptl. Stn., 190, 1-28 (1950).

5. Arle, H.F., Leonard, O.A., and Harris, V.C., "Inactivation of 2,4-D on sweet potato slips with activated carbon". Science, 107, 247-248 (1948).

6. Audus, L.J., "A new soil perfusion apparatus". Nature, 158, 419 (1946).

7. Audus, L.J., "The biological detoxication of 2,4-dichloro phenoxyacetic acid in soil". Plant and Soil, II(1), 31-36 (1949).

8. Audus, L.J., "Biological detoxication of 2,4-dichloro-phenoxyacetic acid in soils: isolation of an effective organism". Nature, 166, 356 (1950).

9. Audus, L.J., "The biological detoxication of hormone herbicides in soil". Plant and Soil, III(2), 170-192 (1951).

10. Audus, L.J., "Plant Growth Regulating Substances", ppxix + 465. Leonard Hill, Ltd., London. 1953.

10a. Audus, L.J., and Quastel, J.H., "Coumarin as a selective phytocidal agent". Nature, 159, 320-324 (1947).

11. Ball, W.S., "Report on 2,4-D experiments in the western states". Proceed. of the Second Ann. Meeting of the North Central States Weed Control Conference. Nov. 1945.

12. Bandurski, R.S., "Spectrophotometric method for the determination of 2,4-dichlorophenoxyacetic acid". Bot. Gaz., 108(3), 446-449 (1947).

13. Bergey et al. "Manual of Determinative Bacteriology". 3rd. Edn. 1930.

14. Biochemical Society Symposia. No.5. "Biological Oxidation of Aromatic Rings". Edited by R.T.Williams.

15. Bouillenne, R., and Bouillenne-Walrand, M., "De l'influence des hormones végétale de synthese sur la croissance de Fusarium vasinfectum". Acad. Roy. Belgique Bull. Cl. Sci., 37(7), 557-566 (1951).

16. Brown, J.W., and Mitchell, J.W., "Inactivation of 2,4-D in soil as affected by soil moisture, temperature, the addition of manure and autoclaving". Bot. Gaz., 109(3), 314-323 (1948).

17. Burr, G.O., and Ashton, F.M., "The effect of soil type on 2,4-D injury to plant cane". Reports of the Hawiian Sugar Technologists 6th. Annual Meeting, Nov. 1948. Honolulu. T.H. pp. 1-15,

18. Burström, H., "Studies on growth and metabolism of roots VI. The relative growth action of different iso-butyric acid derivatives". Physiol. Plantarum, 4(3), 470-485 (1951).

19. Carlyle, R.E., and Thorpe, J.D., "Some effects of ammonium and sodium 2,4-dichlorophenoxyacetates on legumes and the Rhizobium bacteria". Journ. Amer. Soc. Agron., 39(10), 929-936 (1947).

20. Conn, H.J., "A type of bacterium abundant in productive soils but apparently lacking in certain soils of low productivity". Tech. Bull. 138, N.Y. Agric. Exp. Station (1928).

21. Conn, H.J., and Darrow, N.A., "Influence of various non-nitrogenous compounds on the growth of certain bacteria in soils of low productivity". Tech. Bull. 172, N.Y. Agric. Exp. Station (December 1930).

22. Cooper, E.A., and Woodhouse, D.L., "On the relations of the phenols and their derivatives to proteins. A contribution to our knowledge of the mechanism of disinfection. Part IV. The halogen phenols." Biochem. J., 17, 600 (1923).

22a. Crafts, A.S., "A theory of herbicidal action". Science, 108(2795), 85-86 (1948).

23. Crafts, A.S., "Toxicity of 2,4-D in California soils". Hilgardia, 19(5), 141-158 (1949).

24. Crafts, A.S., and Harvey, W.A., "Chemical structure and action of herbicides". Agric. Chem., 5(3), 38-41, 89, (1950).

25. Culler, D.M., "The effects of 2,4-D upon the morphological and physiological characteristics of certain micro-organisms associated with food spoilage". M.S. Thesis. Ohio State University, 1946.

26. De Atley, L.S., "Weed Killers". Agric. Chem., 2(4), 26-29 (1947).

27. De Rose, H.R., "Persistence of some plant growth regulators when applied to the soil in herbicidal treatments". Bot. Gaz., 107(4), 583-589, (1946).

28. De Rose, H.R., and Newman, A.S., "The comparison of the persistence of certain plant growth regulators when applied to soil". Soil Sci. Soc. Amer. Proc. 12. 222-226 (1948).

29. Dubos, R.J., "Inhibition of bacterial growth by auxins". Proc. Soc. Expt. Biol. and Med. 63, 317-319 (1946).

30. Evans, W.C., "Oxidation of phenol and benzoic acid by some soil bacteria". Biochem. J., 41(3), 373-382 (1947).

31. Evans, W.C., Smith, B.S.W., Linstead, R.P., and Elvidge, J.A., "Chemistry of the oxidative metabolism of certain aromatic compounds by micro-organisms". Nature, 168, 772 (1951).

32. Fang, S.C., and Butts, J.S., "Studies in plant metabolism. III.Absorbtion, translocation and metabolism of radio-active 2,4-D in corn and wheat plants". Plant Physiol., 29(1), 56 (1954).

32a. Fang, S.C., Javorski, E.G., Logan, A.V., Freed, V.H., and Butts, J.S., "The absorbtion of 2,4-D (radio-active) and the translocation of C^{14} by bean plants". Arch. Biochem. Biophys., 32, 249-255, (1951).

33. Fawcett, C.H., Ingram, J.M.A., and Wain, R.L., "β-oxidation of w-phenoxyalkylcarboxylic acids in the flax plant". Nature, 170, 887 (1952).

34. Folin, O., and Denis, W., J. Biol. Chem., 22, 305-308 (1915).

34a. Folin, O., and Ciocalteu, V., J. Biol. Chem., 73, 627-650 (1927).

35. Freed, V.H., "Qualitative reaction for 2,4-Dichloro-phenoxyacetic acid. Science, 107, 98-99 (1948).

36. Fromageot, C., et Mayer, A., "Les oxydations en milieu hétérogène et leur intérêt agronomique". Annales Sci. Agron., 43(2), 118-131 (1926).

37. Fults, J.L., and Payne, M.G., "A biometric evaluation of the growth-regulating and herbicidal properties of some organic compounds". J. Amer. Soc. Agron., 39(8), 667-681 (1947).

37a. Fults, J.L., and Payne, M.G., "Some effects of 2,4-D, D.D.T. and Colorado 9 on the bacteria Rhizobium leguminosarum (Frank) in the root nodules of the common bean". Am. J. Botany, 34, 245-248 (1947).

37b. Goldacre, P.L., "Hydrogen peroxide in the enzymic oxidation of heteroauxin". Australian Journ. Sci. Res. Series B. Biol. Sci., 4(3), 293-302 (1951).

38. Gottlieb, S., and Marsh, P.B., "Quantitative determination of phenolic fungicides". Ind. and Eng. Chem. (Analytical Edn.)., 18, (1946).

39. Grabe, E., "Le dosage de l'acide 4-chlore-2-méthyl-phénoxyacétique en composition avec des acides chlorés et nonchlorés". Acta. Chem. Scand., 4(5), 806-9 (1950).

40. Gray, P.H.H., and Thornton, H.G., "Soil bacteria that can decompose certain aromatic compounds". Centralbl. Bakt., 73(1/7), 74-96 (1928).

41. Hamner, C.L., and Tukey, H.B., "Selective herbicidal action of mid-summer and fall applications of 2,4-dichlorophenoxyacetic acid". Bot. Gaz., 106, 232-245 (1944).

42. Hamner, C.L., Moulton, J.E., and Tukey, H.B., "Effect of treating soil and seeds with 2,4-dichlorophenoxyacetic acid on germination and development of seedlings". Bot. Gaz. 107(3), 352-361, (1946).

43. Hamner, C.L., Moulton, J.E., and Tukey, H.B., "Treatment of muck and manure with 2,4-dichlorophenoxy-acetic acid to inhibit germination of weed seeds". Science, 103, 476-477 (1946).

44. Hanks, R.W., "Removal of 2,4-dichlorophenoxyacetic acid and its calcium salt from six different soils by leaching". Bot. Gaz., 108(2), 186-191 (1946).

44a. Hansch, C., Muir, R.M., and Metzenberg, R.L., "Further evidence for a chemical reaction between plant growth-regulators and a plant substrate". Plant Physiol., 26, 812-821 (1951).

45. Hansen, B., "Impurities in technical 'Methoxone' and their influence on plants".
Physiol. Plantarum, 4(4), 667-676 (1951).

46. Happold, F.C., "The oxidation of aromatic rings by micro-organisms in metabolism".
Biochemical Society Symposia No. 5, p85, (1950).

47. Hernandez, T.P., and Warren, G.F., "Factors affecting the rate of inactivation of 2,4-D in peat soil and the rate 2,4-D is leached in different soils".
Proc. Southern Weed Conf., 2, 39 (1949).

48. Hernandez, T.P., and Warren, G.F., "Some factors affecting the rate of inactivation and leaching of 2,4-D in different soils". Proc. Amer. Soc. Hort. Sci., 56, 287-293 (1950).

49. Hey, G.L., and Hope, P.P., "A new theory of the action of plant hormones". Grower,(London), 35(2), 68-69 (1951).

50. Hinshelwood, C.N., "The Chemical Kinetics of the Bacterial Cell". Oxford, Clarendon Press, (1946).

51. Hitchcock, A.E., and Zimmerman, P.W., "Absorption and movement of synthetic growth substances from soil as indicated by the responses of aerial parts".
Contrib. Boyce Thompson Inst., 7, 447-476 (1935).

52. Hoagland, D.R., and Snyder, W.C,, "Nutrition of the strawberry plant under controlled conditions".
Proc. Amer. Soc. Hort. Sci., 30, 288-294 (1933).

53. Holley, R.W., Boyle, F.P., and Hand, D.B., "Studies of the fate of radio-active 2,4-D in bean plants".
Arch. Biochem., 27(1), 143-151 (1950).

54. Holley, R.W., "Studies of the fate of radio-active 2,4-D in bean plants. II. A water-soluble transformation product of 2,4-D". Arch. Biochem. and Biophys., 35(1), 171-175 (1952).

55. Jaworski, E.G., and Butts, J.S., "Studies in plant metabolism. II. The metabolism of C^{14} labelled 2,4-D in bean plants". Arch. Biochem. and Biophys., 38, 207-218 (1952).

56. Jensen, H.L., and Petersen, H.Ingv., "Detoxication of hormone herbicides by soil bacteria".
Nature, 170, 39 (1952).

57. Jensen, H.L., and Petersen, H.Ingv.,
Acta Agriculturae Scandinavica, II. 3 (1952).

57a. Jensen, H.L., and Sörensen, H., "The influence of some organic sulphur compounds and enzyme inhibitors on Nitrosomonas europaea." Acta Agric. Scand., 2, 295-304 (1952).

58. Jones, H.E., "Influence of 2,4-D on nitrate formation in a Prairie soil". J. Amer. Soc. Agron., 40(6),(1948).

59. Jorgensen, C.J.C., and Hamner, C.H., "Weed control in soils with 2,4-D and related compounds and their residual effects under varying environmental conditions". Bot. Gaz.,109(3), 324-333 (1948).

60. Kilby, B.A., "The formation of β-ketoadipic acid by bacterial fission of aromatic rings". Biochem. J., 49, 671 (1951).

61. Kraus, E.J., and Mitchell, J.W., "Growth regulating substances as herbicides". Bot. Gaz., 108(3), 301-350 (1947).

62. Kries, O.H., "Persistence of 2,4-dichlorophenoxyacetic acid in soil in relation to content of water, organic matter and lime". Bot. Gaz., 108(4), 510-525, (1947).

63. Leaper, J.M.F., and Bishop, J.R., "Relation of halogen position to physiological properties in the mono-, di- and trichlorophenoxyacetic acids". Bot. Gaz., 112(3), 250-258 (1951).

64. Lees, H., "A simple automatic percolator". Journ. Agric. Science, 37(1), 27-28 (1947).

65. Lees, H., "The soil percolation technique". Plant and Soil, I(3),(1949).

66. Lees, H., and Quastel, J.H., "Biochemistry of nitrification in soils. I. Kinematics of, and the effects of poisons on, soil nitrification as studied by a soil perfusion technique". Biochem. J., 40, 803-828 (1946).

67. Letourneau, D., and Krog, N., "The use of chromotropic acid for the quantitative determination of 2,4-D". Plant Physiol., 27(4), 822-827 (1952).

68. Lewis, R.W., and Hamner, C.L., "Effect of 2,4-D on some micro-organisms". Mich. Agric. Expt, Sta. Quart. Bull., 29, 112-114 (1946)

68a. Lichstein, and Umbreit, J. Biol. Chem., 170, 329,423, 1947.

69. Lochhead, A.G., and Taylor, C.B., "Qualitative studies in soil micro-organisms". Can. J. Research, C, 16, 152-173 (1938).

70. Lochhead, A.G., and Thexton, R.H., "Vitamin B_{12} as a growth factor for soil bacteria". Nature, 167, 1034 (1951).

71. Loustalot, A.J., and Ferrer, R., "The effect of temperature on the persistence of sodium pentachlorphenate in the soil". Proc. Southern Weed Conf., 2, 55-58 (1949).

72. Loustalot, A.J., and Ferrer, R., "The effect of some environmental factors on the persistence of sodium pentachlorphenate in the soil". Proc. Amer. Soc. Hort. Sci., 56, 294-298 (1950).

73. Lucas, E.H., and Hamner, C.L., "Inactivation of 2,4-D by absorption on charcoal". Science, 105, 340 (1947).

74. Manil, P., and Straszewska, Z., "Action de l'acide dichlorophénoxyacétique (2,4-D) sur les moisissures". Compt. Rende. Soc. Biol.,144(3/4), 313-315 (1950).

75. Mann,P.J.G., and Quastel, J.H., "Manganese metabolism in soils". Nature, 158, 154 (1946).

76. Marcelli, E., "Effetti sulla microflora del terreno del 2,4-D ad altedosi". Tabaccs, 54,(616-617), 370-374 (1950).

77. Martin, J.P., "The hormone weedkiller, 2,4-D". California Citrograph, 31(7), 248-264 (1946).

78. McRae, D.H., and Bonner, J., "Di-ortho substituted phenoxyacetic acids as anti-auxins". Plant Physiol.,27(4), 834-838 (1952).

79. Meuli, L.J., Theigs, B.J., and Lynn, G.E., "The zinc salt of 2,4,5-trichlorphenol as a seed fungicide". Phytopath., 37(7), 474-480 (1947).

80. Milton, R.F., and Waters, W.A., "Methods of Quantitative Micro-analysis". Arnold. 1949.

81. Mitchell, J.W., and Marth, P.C., "Germination of seeds in soil containing 2,4-Dichlorophenoxyacetic acid". Bot. Gaz., 107(3), 408-416 (1946).

82. Moewus, F., "Ein neuer quantitativer Test für pflanzliche Wuchstoffe." Naturwiss., 35(4), 124-125 (1948).

83. Moewus, F., "Gebundener und freier Wuchsstoff in fleischigen Fruchten". Planta, 37(3), 413-430 (1949).

84. Muir, R.M., Hansch, C.H., and Gallup, A.H., "Growth regulation by organic compounds". Plant Physiol.,24(3), 359-366 (1949).

85. Muir, R.M., and Hansch, C., "The relationship of structure and plant-growth activity of substituted benzoic and phenoxyacetic acids". Plant Physiol., 26(2), 369-374 (1951).

85a. Muir, R.M., and Hansch C., "On the mechanism of action of growth regulators". Plant Physiol., 28(2), 218-232 (1953).

86. Muzic, T.J., Loustalot, A.J., and Cruzado, H.J., "Movement of 2,4-D in soil". Agron. Journ., 43(3), 149-150 (1951).

87. Newman, A.S., "The effect of certain plant growth regulators on soil micro-organisms and microbial processes". Soil Sci. Soc. Amer. Proc., 12, (1947).

87a. Newman, M.S., Fones, W., and Renoll, M., "New compounds as plant growth regulators". J. Am. Chem. Soc., 69, 718-723 (1947).

88. Newman, A.S., and Norman, A.G., "Effect of soil micro-organisms on the persistence of plant growth regulators in the soil". Journ. Bact., 54, 37-38 (1947).

89. Newman, A.S., and Thomas, J.R., "Decomposition of 2,4-dichlorophenoxyacetic acid in soil and liquid media". Soil Sci. Soc. Amer. Proc., 14, 160-164 (1950).

90. Newman, A.S., Thomas, J.R., and Walker, R.L., "Dissapearance of 2,4-D and 2,4,5-T from soil". Soil Sci. Soc. Amer. Proc., 16(1), 21-24 (1952).

91. Norman, A.G., and Newman, A.S., "The persistence of herbicides in soils". Proc. North-eastern States Weed Control Conference, New York City, Jan.1950. pp.7-12.

92. Nutman, P.S., Thornton, H.G., and Quastel, J.H., "Inhibition of plant growth by 2,4-dichlorophenoxyacetic acid and other plant growth substances". Nature, 155, 498-500 (1945).

93. Offord, H.R., "Rapid estimation of the phytocidal activity of chemicals". Science, 103, 474-476 (1946).

94. Ordal, E.J., "Relative germicidal action of some halogenated phenols and their phenolates". Proc. Soc. Exptl. Biol. and Med., 47(2), 387-389 (1941).

94a. Osborne, D.J., and Wain, R.L., Proc. IInd. Int. Congr. Crop Protection, London, 1949, p.325.

95. Paleg, L.G., and Muir, R.M., "Surface activity as related to physiological activity of plant growth-regulators". Plant Physiol., 27(2), 285-292 (1952).

96. Payne, M.G., and Fults, J.L., "Some effects of 2,4-D, D.D.T. and Colorado 9 on root nodulation in the common bean". Journ. Amer. Soc. Agron., 39, 52-55 (1947).

97. Petersen, H.Ingv., "Forsøg med kemiske midler mod ukrudt i varsaeds - og stubmarker". Tideskrift f. Planeavl, 53, 678-708 (1950).

98. Poole, E.A., and Hinshelwood, C.N., "Physiochemical aspects of bacterial growth. VI. The influence of toxic substances on growth rate, stationary population and fermentation reactions of Bact. lactis aerogenes. VII. Influence of phenol on the death rate of Bact. lactis aerogenes". Journ. Amer. Chem. Soc., 62, VI. 1565-1572, VII. 1573, (1940).

99. Porteous and Williams, "Determination of phenols using 2,6-dichloroquinone chloroimide reagent". Biochem. Journ., 44, (1949).

100. Quastel, J.H., and Lees, H., Chem. and Ind., 26, 238 (1944).

101. Rudolph, W., "Wuchstoffe und Antiwuchstoffe". Naturwiss. Rundschau, 3(7), 312-314 (1950).

102. Ready, D., and Grant, V.Q., "A rapid sensitive method for determination of low concentrations of 2,4-D in aqueous solution". Bot. Gaz., 109(1), 39-44 (1947).

103. Shanmuga Sundaram, E.R.B., Tirunarayanan, M.O., and Sarma, P.S., "The relationship between biotin and tryptophane metabolism studied in Neurospora crassa". Biochem. Journ., 58, 469 (1954).

104. Schmidt, E.L., "Soil micro-organisms and plant growth-substances. I. Historical. Soil Science, 71(2), 129-140 (1951).

105. Sen Gupta, N.N., "Dephenolization in soil". Journ. Agric. Sci., 11, 136-158 (1921).

106. Sjoberg, B., "Determination of 4-chloro,2-methylphenoxy-

106.cont. -acetic acid by infra-red spectrophotometry". Acta. Chem. Scand., 4(5), 798-805 (1950).

107. Slade, R.E., Templeman, W.G., and Sexton, W.A., "Plant growth substances as selective weed killers. Differential effect of plant growth substances on plant species". Nature, 155, 497-498 (1945).

108. Sleeper, B.P., and Stanier, R.Y., "The bacterial oxidation of aromatic compounds. I. Adaptive patterns with respect to polyphenolic compounds". Journ. Bact., 59(1), (1950).

109. Sleeper, B.P., Tsuckida, M., and Stanier, R.Y., "The bacterial oxidation of aromatic compounds. II. The preparation of enzymatically active dried cells and the influence thereon of prior patterns of adaptation". Journ. Bact., 59(1), (1950).

109a. Smith, F.E., and Collins, T., "The preparation of the optical isomers of α-(2,4-dichlorophenoxy)propionic acid and their growth regulating activity". Journ. Sci. of Food and Agric., 6, 248-249 (1952).

110. Smith, M.S., Wain, R.L., and Wightman, F., "Studies of plant growth regulating substances. V. Steric factors in relation to mode of action of certain aryloxyalkyl-carboxylic acids." Ann. Appl. Biol., 39(3), 295-307 (1952).

111. Smith, N.R., Dawson, V.T., and Wenzel, M.E., "The effect of certain herbicides on soil micro-organisms". Soil Sci. Soc. Amer. Proc., 10, 197-201 (1946).

112. Smith, M.S., and Wain, R.L., "The plant growth regulating activity of dextro- and laevo- α-(2-naphthoxy-) propionic acid". Proc. Roy. Soc. B., 139, (1951).

113. Snell, D.S., and Snell, C.T., "Colorimetric Methods of Analysis". D. Van Nostrand Co. Inc., New York, 1937.

114. Stanier, R.Y., "Studies on the mechanism of enzymic degradation of protocatechuic acid". (No.IV. in series). Journ. Bact., 59(4), (1950).

115. Stanier, R.Y., "Problems of bacterial oxidative metabolism". Bact. Rev., 14, 179 (1950).

116. Stanier, R.Y., Sleeper, B.P., Tsuckida, M., and Macdonald, D.L., "The bacterial oxidation of aromatic compounds. III. The enzymatic oxidation of catechol and protocatechuic acid to β-keto-adipic acid". Journ. Bact., 59(2), (1950).

117. Stapp, C., and Freter, R., "Untersuchungen über die Wirkung von 2,4-D im Boden". Phytopath. Zeitschr., 19(1), 20-33 (1952).

118. Stevenson, E.C., and Mitchell, J.W., "Bacteriostatic and bacteriocidal properties of 2,4-dichlorophenoxyacetic acid". Science, 101, 642-644 (1945).

119. Swanson, C.P., "A simple bio-assay method for the determination of low concentrations of 2,4-D in aqueous solutions". Bot. Gaz., 107(4), 507-509 (1946).

120. Synerholm, M.E., and Zimmerman, P.W., "Preparation of a series of w-(2,4-dichlorophenoxy)aliphatic acids and some related compounds with a consideration of their biochemical role as plant growth regulators". Contr. Boyce Thompson Inst., 14(7), 369-382 (1947).

121. Synerholm, M.E., and Zimmerman, P.W., "The preparation of some substituted phenoxyalkyl-carboxylic acids and their properties as growth substances". Contrib. Boyce Thompson Inst., 14(2), 91-104 (1945).

122. Taylor, C.B., "Nature of the factor in soil-extract responsible for bacterial growth stimulation". Nature, 168, 115 (1951).

123. Taylor, D.L., "Growth of field crops in soil treated with chemical growth regulators". Bot. Gaz., 108(3), 432-445 (1947).

123a. Thayer and Horowitz, J. Biol. Chem., 192, 755 (1951).

124. Thimann, K.V., "The role of ortho substitution in the synthetic auxins". Plant Physiol., 27(2), 392-404 (1952).

125. Thompson, H.E., Swanson, C.P., and Norman, A.G., "New growth-regulating compounds. I. Summary of growth inhibitory activities of some organic compounds as determined by three tests". Bot. Gaz., 107(4), 476-507 (1946).

126. Van Overbeek, J., and Velez, I., "Use of 2,4-dichlorophenoxyacetic acid as a selective herbicide in the tropics". Science, 103, 472-473 (1946).

127. Veldstra, H., "The relation of chemical structure to biological activity in growth substances". Ann. Rev. Plant Physiology, p.424 (1953).

128. Wain, R.L., "Chemical aspects of plant growth regulating activity". Ann. Appl. Biol., 36, 558 (1949).

129. Walker, N., "Preliminary observations on the decomposition of chlorophenols in soil". Plant and Soil, V(2), 194-204 (1954).

130. Warshowsky, B., and Schantz, E.J., J. Anal. Chem., 22, 460 (1950).

131. Weaver, R.J., "Contratoxification of plant growth regulators in soils and on plants". Bot. Gaz., 109(3), 276-300 (1948).

132. Weaver, R.J., "Reaction of certain plant growth regulators with ion exchangers". Bot. Gaz., 109(1), 72-84 (1948).

133. Weintraub, R.L., Brown, J.W., Fields, M., and Rohan, J., "Metabolism of 2,4-D. I. $C^{14}O_2$ production by bean plants treated with labelled 2,4-D." Plant Physiol., 27(2), 293-301 (1952).

134. Weintraub, R.L., Brown, J.W., Fields, M., and Rohan, J., "Production of $C^{14}O_2$ from labelled 2,4-D by plants". Amer. Journ. Bot., 37(8), 682 (1950).

135. Weintraub, R.L., Yeatman, J.N., Lockhart, J.A., Reinhart, J.H., and Fields, M., "Metabolism of 2,4-D. II. Metabolism of the side-chain by bean plants". Arch. Biochem. and Biophys., 40(2), 277-285 (1952).

136. Wolfe, W.C., Wood, J.W., Doukas, H.M., Klipp, L.W., and Mitchell, J.W., "Synthetic plant growth regulators. I. 2,4-dichloro-5-iodophenoxyacetic acid and derivatives. II. Radio-active labelled 2,4-dichloro-5-iodo^{131}phenoxy-acetic acid and derivatives". Journ. Org. Chem., 14(5), 900-906 (1949).

137. Wood, J.W., and Fontaine, T.D., "Synthetic plant growth regulators. III. 2,4-dichlorophenoxyacetyl derivatives of amino-acids". Journ. Org, Chem., 17(6), 891-896 (1952)

138. Worth, W.A.Jr., and McCabe, A.M., "Differential effects of 2,4-D on aerobic, anaerobic and facultative anaerobic micro-organisms". Science, 108, 16-18 (1948).

139. Zimmerman, P.W., "Present status of plant hormones". Indust. and Eng. Chem., Indust. Edn., 35(5), 596-601 (1943).

140. Zimmerman, P.W., and Hitchcock, A.E., "Substituted phenoxy and benzoic acids as growth substances and the relation of structure to physiological activity". Contrib. Boyce Thompson Inst., 12(5), 321-343 (1942).

141. Zobell, C.E., "Action of micro-organisms on hydrocarbons". Bact. Rev., 10, 1 (1946).

142. Zobell, C.E., and Michener, H.D., "A parodox in the adaptation of marine bacteria to hypotonic solutions". Science, 87, 328 (1938).

Table I. Standard Curve for Phenoxyacetic Acid (POAA).

Herbicide Concentration(ppm.).	Longest Roots(mm.).	Longest Root Length as Percentage Mean Control
1,000	4; 5;	4; 5;
100	21; 25;	20; 24;
10	52; 66;	51; 64;
1	86; 101;	84; 98;
0.1	94; 107;	91; 104;
0.01	95; 111;	92; 108;
Controls	98; 108;	95; 105;

By interpolation, the concentration of POAA needed to produce 50% inhibition of cress root growth was 16.5 ppm. approximately. This degree of inhibition was produced by 0.03 ppm. 2,4-D. The Relative Toxicity of POAA is, therefore, 0.03 X 100 / 16.5 = 0.2 approximately. (See p.44).

Results plotted on Graph I.

Table II. Standard Curve for 2-chlorophenoxyacetic acid (2-CPA).

Conc. of 2-CPA (ppm).	Lengths of longest roots at each concentration. 1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	Mean.	Mean as % mean control.	Stand. Dev.±	S.D. as % mean control.
30.0,	3,	3,	4,	4,	4,	5,	5,	5,	5,	6,	4.4,	6.1,	0.92,	1.3,
10.0,	9,	9,	10,	11,	11,	11,	11,	12,	13,	15,	11.2,	15.6,	1.72,	2.4,
3.0,	16,	18,	20,	21,	23,	24,	27,	29,	30,	31,	23.9,	33.3,	4.95,	6.9,
1.0,	24,	26,	29,	30,	31,	34,	36,	38,	40,	43,	33.1,	46.1,	5.86,	8.2,
0.3,	36,	38,	40,	41,	45,	46,	48,	53,	58,	61,	46.6,	64.9,	8.00,	11.1,
0.1,	50,	52,	57,	59,	64,	67,	71,	73,	79,	81,	65.3,	90.9,	10.2,	14.2,
0.03,	55,	57,	65,	66,	69,	70,	74,	75,	85,	87,	70.2,	97.7,	10.1,	14.1,
0.01,	60,	63,	65,	67,	72,	72,	75,	82,	85,	86,	72.7,	101.2,	8.76,	12.2,
0.003,	59,	62,	64,	67,	71,	74,	77,	79,	84,	86,	72.3,	100.6,	8.81.	12.3,
Controls in distilled water.	58,	58,	60,	62,	64,	65,	67,	69,	70,	74,				
	74,	76,	78,	82,	83,	83,	84,	86,			71.83,	100.0,	9.21,	12.8,

Relative Toxicity of 2-CPA is 0.03 x 100 / 0.8 = 4.0 (approximately).

Results plotted on Graph II.

Table III. Standard Curve for 3-chlorophenoxyacetic acid.

Herbicide Concentration(ppm.).	Longest Roots(mm.).	Longest Root Length as Percentage Mean Control.
100	4; 7;	4; 7;
10	17; 24;	18; 26;
1	34; 51;	36; 54;
0.1	69; 94;	74; 100;
0.01	96; 105;	102; 112;
0.001	86; 99;	92; 106;
0.0001	88; 98;	94, 104;
Controls	90; 98;	96; 104;

Relative Toxicity of 3-CPA is 0.03 x 100 / 0.8 = 4.0(approx.)

Results plotted on Graph III.

Table IV. Standard Curve for 4-chlorophenoxyacetic acid (4-CPA).

Conc. of 4-CPA (ppm).	Lengths of longest roots at each concentration(mm). 1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	Mean.	Mean as % mean control.	Stand. Dev.±	S.D.as % mean control.
1.0	2,	3,	3,	3,	4,	4,	4,	5,	5,	6,	3.9,	4.6,	1.14,	1.3,
0.3	6,	7,	8,	9,	9,	10,	10,	11,	12,	12,	9.4,	11.1,	1.91,	2.2,
0.1	16,	18,	19,	21,	21,	22,	26,	29,	33,	36,	24.1,	28.3,	6.33,	7.5,
0.03	25,	30,	33,	37,	41,	43,	45,	50,	54,	62,	42.0,	49.5,	10.8,	12.7,
0.01	52,	55,	59,	64,	68,	75,	80,	86,	90,	96,	72.5,	85.4,	14.5,	17.1,
0.003	62,	66,	69,	76,	80,	84,	87,	92,	96,	98,	81.0,	95.5,	12.0,	14.1,
0.001	68,	74,	77,	81,	84,	87,	90,	94,	99,	101,	85.5,	100.8,	10.3,	12.1,
0.0003	74,	77,	81,	83,	86,	88,	91,	94,	98,	104,	87.6,	103.3,	8.9,	10.5,
0.0001	70,	72,	75,	83,	88,	90,	94,	95,	98,	105,	87.0,	102.5,	11.1,	13.1,
0.00003	70,	72,	81,	81,	85,	88,	89,	91,	94,	96,	84.7,	99.8,	8.3,	9.8,
0.00001	70,	72,	76,	79,	84,	89,	92,	93,	96,	101,	85.2,	100.4,	10.1,	11.9,
Controls	73,	73,	74,	75,	77,	77,	79,	79,	80,	81,				
	83,	84,	87,	88,	88,	89,	91,	92,	95,	96,				
	103,	104,									84.91,	100.00,	9.0,	10.6,

Relative Toxicity of 4-CPA is 0.03 x 100 / 0.03 = 100. Results plotted on Graph IV.

Table V. Standard Curve for 4-iodophenoxyacetic acid (4-IPA).

Conc. of 4-IPA (ppm).	Lengths of longest roots at each concentration. 1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	Mean.	Mean as % mean control.	Stand. Dev.±	S.D.as % mean control.
100.0	1.0,	1.5,	1.5,	1.5,	2.0,	2.0,	2.0,	2.0,	2.5,	3.0,	1.9,	2.4,	0.58,	0.73,
10,0	4,	6,	6,	7,	7,	7,	8,	9,	10,	11,	7.5,	9.4,	1.96,	2.5,
3,0	10,	12,	12,	14,	16,	16,	16,	17,	19,	21,	15.4,	19.3,	2.67,	3.4,
1.0	26,	26,	28,	28,	32,	36,	38,	40,	45,	50,	34.9,	43.8,	7.9,	9.9,
0.3	36,	37,	40,	46,	48,	49,	58,	63,	68,	69,	51.4,	64.5,	11.8,	14.8,
0.1	62,	63,	66,	68,	73,	76,	76,	85,	89,	96,	75.4,	94.6,	10.9,	13.7,
0.03	67,	69,	70,	75,	80,	82,	84,	88,	94,	96,	80.5,	101.0,	9.7,	12.2,
0.01	70,	72,	74,	78,	79,	83,	85,	90,	91,	98,	82.0,	102.9,	8.6,	10.8,
0.001	66,	70,	73,	76,	77,	80,	84,	88,	90,	94,	79.8,	100.1,	9.1,	11.4,
Controls in distilled water.	66,	66,	68,	70,	72,	72,	74,	78,	80,	81,				
	82,	83,	86,	88,	89,	90,	94,	96,			79.72,	100.0,	9.3,	11.7,

Relative Toxicity of 4-IPA is 0.03 x 100 / 0.63 = 5 (approximately).

Results plotted on Graph V.

Table VI. Standard Curve for 2,4-dichlorophenoxyacetic acid (2,4-D).

Conc. of 2,4-D (ppm).	Lengths of longest roots at each concentration. 1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	Mean.	Mean as % mean control.	Stand. Dev.±	S.D. as % mean control.
1.0	1.0,	1.5,	2.0,	2.0,	2.5,	3.0,	3.0,	3.0,	3.5,	3.5,	2.5,	3.5,	0.81,	1.1,
0.3	4.5,	5.0,	5.5,	6.5,	7.0,	7.0,	7.5,	8.5,	9.5,	10.5,	7.2,	9.9,	1.83,	2.5,
0.1	10,	11,	12,	13,	15,	16,	18,	18,	20,	21,	15.4,	21.3,	3.65,	5.1,
0.03	23,	25,	29,	32,	34,	37,	39,	43,	48,	50,	36.0,	49.8,	8.7,	12.4,
0.01	41,	45,	48,	55,	58,	63,	66,	70,	76,	83,	60.5,	83.8,	13.0.	18.0,
0.003	54,	58,	59,	68,	71,	73,	77,	81,	87,	91,	71.9,	99.6,	11.8,	16.3,
0.001	60,	63,	68,	70,	70,	74,	76,	80,	86,	93,	74.0,	102.6,	9.6,	13.4,
0.0003	59,	62,	64,	69,	72,	72,	73,	80,	86,	88,	72.5,	100.5,	9.8,	12.8,
0.0001	59,	62,	65,	70,	72,	73,	78,	81,	84,	88,	73.0,	101.2,	9.8,	13.6,
Controls in distilled water.	56,	57,	58,	61,	62,	67,	68,	70,	71,	71,				
	74,	77,	81,	82,	84,	85,	86,	90,			72.22,	100.0,	10.5,	14.6,

Relative Toxicity of 2,4-D by definition is 100.

Results Plotted on Graph VI.

Table VII. Standard Curve for 2,5-dichlorophenoxyacetic acid.

Herbicide Concentration(ppm.).	Longest Roots(mm.).		Longest Root Length as Percentage Mean Control.	
100	2;	5;	2;	5;
10	6;	11;	6;	11;
1	23;	38;	24;	40;
0.1	51;	83;	54;	88;
0.01	89;	108;	94;	114;
0.001	85;	103;	90;	108;
Controls	92;	98;	97;	103;

Relative Toxicity of 2,5-D is 0.03 x 100 / 0.355 = 8.5 (approx.)

Results plotted on Graph VII.

Table VIII. Standard Curve for 3,4-dichlorophenoxyacetic acid.

Herbicide Concentration(ppm.).	Longest Roots(mm.).		Longest Root Length as Percentage Mean Control.	
100	1;	2;	1;	2;
10	6;	8;	6;	8;
1	30;	38;	30;	38;
0.1	82;	96;	82;	96;
0.01	100;	110;	100;	110;
0.001	94;	108;	94;	108;
Controls	96;	104;	96;	104;

Relative Toxicity of 3,4-D is 0.03 x 100 /0.55= 0.55(approx.)

Results plotted on Graph VIII.

Table IX. Standard Curve for 2,4,5-trichlorophenoxyacetic acid.

Herbicide Concentration(ppm.).	Longest Roots(mm.).		Longest Root Length as Percentage Mean Control.	
100	1;	1;	1;	1;
10	3;	3;	3;	3;
1	14;	16;	14.5;	16.5;
0.1	59;	65;	61.5;	68;
0.01	87;	98;	91;	102;
0.001	92;	100;	96;	104;
Controls	93;	99;	97;	103;

Relative Toxicity of 2,4,5-T is 0.03 x 100 / 0.175 = 17 (app.)

Results plotted on Graph IX.

Table X. Standard Curve for 4-chloro,2-methylphenoxyacetic acid (MCPA).

Conc. of MCPA (ppm).	Lengths of longest roots at each concentration. 1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	Mean.	Mean as % mean control.	Stand. Dev.±	S.D. as % mean control.
1.0	1,	1,	2,	2,	2,	2,	3,	4,	4,	4,	2.5,	2.9,	1.12,	1.3,
0.3	3,	3,	4,	4,	6,	6,	6,	7,	8,	8,	5.5,	6.3,	1.70,	1.9,
0.1	8,	10,	12,	15,	17,	18,	18,	18,	19,	19,	15.4,	17.5,	3.8,	4.3,
0.03	23,	26,	29,	32,	37,	39,	43,	45,	49,	54,	37.7,	42.9,	9.7,	11.0,
0.01	38,	42,	45,	48,	52,	55,	59,	64,	68,	74,	54.5,	62.0,	11.1,	12.7,
0.003	60,	64,	67,	71,	75,	81,	88,	94,	100,	104,	80.4,	91.6,	14.7,	16.8,
0.001	72,	74,	77,	82,	86,	90,	94,	98,	100,	104,	87.7,	99.9,	10.7,	12.2,
0.0003	71,	74,	77,	82,	88,	92,	96,	99,	101,	106,	88.6,	100.9,	11.5,	13.1,
0.0001	72,	74,	77,	82,	87,	91,	95,	97,	102,	104,	88.1,	100.3,	11.9,	12.5,
0.00003	69,	74,	77,	85,	87,	90,	94,	97,	101,	103,	87.7,	99.7,	11.0,	12.5,
0.00001	72,	75,	78,	83,	87,	91,	93,	97,	100,	105,	88.1,	100.3,	10.5,	11.9,
Controls in distilled water.	67,	70,	74,	76,	78,	80,	81,	82,	82,	86,	87.82,	100.0	11.2,	12.7,
	87,	89,	91,	91,	93,	94,	95,	98,	101,	103,				
	105,	109,												

Relative Toxicity of 4-chloro,2-methylphenoxyacetic acid is 0.03 x 100 / 0.022 = 140 (app.)

Results Plotted On Graph X.

Table XI. Standard Curve for 2,4-dimethylphenoxyacetic acid (2,4-DM).

Conc. of 2,4-DM (ppm).	Lengths of longest roots at each concentration. 1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	Mean.	Mean as % mean control.	Stand. Dev. ±	S.D. as % mean control.
10.0	1.0,	1.0,	2.0,	2.5,	3.0,	3.0,	3.0,	3.0,	3.5,	4.0,	2.6,	3.1,	0.94,	1.13,
3.0	5,	5,	5,	6,	6,	7,	7,	7,	10,	12,	7.0,	8.4,	2,19,	2.64,
1.0,	9,	10,	12,	13,	14,	14,	15,	16,	16,	20,	13.9,	16.8,	3.01,	3.63,
0.3	19,	19,	24,	25,	29,	30,	34,	37,	40,	44,	30.1,	36.3,	8.2,	9.9,
0.1	35,	39,	42,	47,	52,	53,	58,	62,	69,	72,	52.9,	63.9,	11.8,	14.2,
0.03	49,	56,	61,	67,	69,	72,	75,	80,	85,	88,	70.2,	84.6,	11.9,	14.6,
0.01	66,	68,	70,	74,	78,	80,	84,	88,	90,	96,	79.4,	95.9,	9.5,	11.5,
0.003	69,	70,	72,	78,	82,	86,	88,	91,	94,	97,	82.7,	99.8,	9.6,	11.6,
0.001	70,	73,	76,	78,	78,	85,	88,	88,	90,	94,	82.0,	99.0,	7.6,	9.2,
Controls in distilled water.	71,	71,	73,	74,	76,	76,	78,	79,	82,	83,				
	85,	86,	88,	89,	90,	94,	96,	100,			82.83,	100.0,	8.62,	10.4,

Relative Toxicity of 2,4-DM is 0.03 x 100 / 0.17 = 18 (approximately).

Results plotted on Graph XI.

Table XII. Standard Curve for ∝-4-chlorophenoxypropionic acid.

Herbicide Concentration(ppm.).	Longest Roots(mm.).		Longest Root Length as Percentage Mean Control.	
10	2;	3;	2;	3;
1	11;	12;	11;	12;
0.1	37;	48;	37.5;	48.5;
0.01	74;	96;	74;	96;
0.001	95;	105;	96;	106;
0.0001	102;	110;	103;	111;
Controls	95;	103;	96;	104;

Relative Toxicity of ∝-4-CPP is 0.03 x100 / 0.06 = 50 (approx.)

Results plotted on **Graph XII.**

Table XIII. Standard Curve for ∝-2,4-dichlorophenoxypropionic acid (∝-2,4-DCPP).

Conc. of ∝-2,4-DCPP (ppm).	Lengths of longest roots at each concentration. 1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	Mean.	Mean as % mean control.	Stand. Dev. ±	S.D. as % mean control.
1.0	2.5,	3.0,	3.0,	3.5,	3.5,	4.0,	4.5,	5.0,	5.5,	5.5,	4.0,	4.9,	1.03,	1.2.
0.3	11,	12,	12,	13,	13,	14,	15,	16,	17,	18,	14.1,	17.3,	2.2.	2.7,
0.1	14,	15,	17,	18,	20,	22,	24,	26,	29,	32,	21.7,	26.5,	5.7,	7.0,
0.03	33,	36,	41,	45.	49,	52,	56,	62,	66,	67.	50.7,	62.0,	11.5.	14.0,
0.01	54,	58,	61,	64,	69,	71,	74,	80,	83,	85,	69.9.	85.4.	10.1,	12.4,
0.003	72,	73,	75,	76.	79,	84,	87,	88,	92.	94.	82.0,	100.2,	7.6.	9.3,
0.001	68,	71,	75,	78,	79.	81.	84,	86,	90,	92,	80.4,	98.3,	7.4,	9.1,
0.0003	71,	72,	73,	74,	77,	80,	84,	86,	88,	88,	79.1,	97.0,	6.2,	7.6,
0.0001	68,	71,	75,	76,	78,	80,	85,	88,	92,	93,	80.6,	98.5,	8.2,	10.0,
0.00003	70,	72,	75,	77,	81,	83,	86.	88,	88,	91,	81.1,	99.5,	6.9,	8.5,
0.00001	70,	73,	75,	77,	81,	83,	86,	89,	90,	92,	81.6,	99.8,	7.3,	8.9,
Controls in distilled water.	68,	70,	70,	72,	74,	75,	76,	78,	78,	80,				
	80,	81,	83,	83,	85,	85,	86,	86,	87,	90,				
	92,	94,	95,	96.							81.83.	100.0.	7.97.	10.0.

Relative Toxicity of ∝-2,4-dichlorophenoxypropionic acid is 0.03 x 100 / 0.045 = 67 (approx.)

Results plotted on Graph XIII.

Table XIV. Standard Curve for 2-chlorphenol.(2-CP).

Table XIVa. Standard Curve for 4-chlorphenol.(4-CP).

Table XIVb. Standard Curve for 2,4-dichlorphenol.(2,4-DCP).

Phenol Concentration in parts per million.	Colourimeter Reading (E.E.L. Instrument.).		
	XIV. (2-CP).	XIVa. (4-CP).	XIVb. (2,4-DCP).
10	9.3	7.4	5.2
20	18.0	13.8	10.0
30	27.7	23.2	16.0
40	37.1	30.6	19.3
50	45.3	38.3	23.8
60	55.7	45.0	30.0
70	63.2	50.7	35.0
80	73.4	59.4	40.0
90	81.4	68.3	46.1
100	93.2	72.9	50.2
110	98.5	84.5	53.7
120	110.0	89.2	60.2

Table XV. Standard Curve for 4-chlorphenol (4-CP).

Table XVa. Standard Curve for 2,4-dichlorphenol (2,4-DCP).

Phenol Concentration in parts per million.	Colourimeter Reading (Unicam Instrument.).			
	XV. (4-CP).		XVa. (2,4-DCP).	
	Percentage Transmission.	Optical Density.	Percentage Transmission.	Optical Density.
10	79.5	0.100	83.2	0.078
20	64.2	0.193	74.6	0.127
30	50.2	0.300	61.1	0.212
40	39.4	0.405	53.4	0.272
50	32.4	0.490	45.6	0.340
60	25.2	0.599	40.3	0.393
70	20.3	0.693	33.9	0.468
80	15.6	0.808	29.6	0.528
90	12.3	0.910	24.7	0.608
100	10.0	1.000	22.1	0.652

$$\text{Optical Density} = \log. \frac{100}{\% \text{ transmission.}}$$

4-chlorphenol concentration (ppm.) = Optical Density x 100.

2,4-dichlorphenol concentration (ppm.) = Optical Density x 150

Table 16. Direct perfusion of phenoxyacetic acid.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 100 ppm., as percentage of mean control.
D. Indicated POAA concentration in the perfusate (ppm.).

A.	B.	C. C.	D.	
0	102.9	24, 27,	80	Perfusion was started on 7/7/53
3	102.9	22, 24,	90	with 50 gm. of soil (2 to 4 mm.,
6	102.9	20, 21,	110	Sussex Lodge soil, dried
9	102.9	22, 23,	90	January 1953,) and 250 ml. of
12	102.9	24, 26,	80	100 ppm. POAA solution. First
15	102.9	23, 26,	90	sample taken after 1 hr.
27	99.4	19, 20,	120	perfusion.
33	96.4	21, 22,	105	
39	96.1	59, 59,	10	
45b	96.4	55, 55,	10	

Perfuser drained and refilled with 250 ml. of 100.ppm. POAA.

45a	96.4	29, 33,	50	Sampled after 1 hr. perfusion.
48	95.2	41, 42,	30	
51,	99.1	42, 45,	25	
54	99.1	98,103,	<1	
57	97.3	91, 94,	<1	

Table 16a. Direct perfusion of phenoxyacetic acid.

Key to columns: as in Table 16, above.

A.	B.	C. C.	D.	
0	102.9	35, 36,	40	Perfusion was started on 7/7/53
3	102.9	25, 37,	60	Details as for perfuser 16,
6	102.9	34, 35,	55	in table above. First sample
9	102.9	33, 38,	45	taken after 1 hr. Initial
12	102.9	34, 37,	40	perfusate strength was only
15	102.9	32, 37,	45	50 ppm. POAA.
27	99.4	34, 39,	40	
33	96.4	55, 57,	10	
39	96.1	55, 72,	7	
45b	96.4	65, 68,	6	

Perfuser drained and refilled with 250 ml. of 100 ppm. POAA.

45a	96.1	27, 29,	70	54	99.1	106,115,	<1
48	95.2	30, 30,	60	57	97.3	91, 94,	<1
51	99.1	42, 43,	30				

Table 17. Direct perfusion of 2-chlorophenoxyacetic acid.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.1 ppm. as percentage of mean control.
D. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
E. Longest roots at nominal concentration of 10 ppm. as % m.c.
F. Indicated 2-CPA concentration in the perfusate (ppm.).

Perfusion started on 20/12/51 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried June 1951,) and 250 ml. of 100 ppm. 2-CPA solution. First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	52.3	73,	78,	84,	88,	31,	34,	38,	44,	16,	18,	100
10	52.3	75,	82,	86,	99,	34,	36,	42,	52,	17,	21,	90
13	81.3	79,	80,	83,	90,	27,	36,	44,	53,	15,	17,	100
16	77.7	82,	84,	90,	99,	40,	45,	46,	52,	19,	21,	70
19	72.0	72,	74,	74,	78,	39,	45,	45,	65,	15,	17,	80
22	59.0	88,	90,	94,	102,	63,	68,	73,	75,	12,	14,	110
25	59.7	82,	90,	117,	117,	33,	33,	55,	64,	12,	12,	100
28	47.2	62,	76,	85,	89,	36,	47,	59,	66,	15,	25,	80
31	51.2	76,	78,	80,	109,	41,	45,	45,	53,	18,	22,	70
33	43.2	97,	102,	102,	112,	60,	60,	70,	79,	14,	23,	70
36	58.1	79,	86,	91,	126,	38,	41,	48,	62,	16,	26,	70
39	74.6	64,	64,	69,	69,	32,	32,	45,	53,	13,	16,	110
42	67.0	76,	86,	88,	104,	36,	37,	52,	54,	14,	16,	80
45	57.1	103,	107,	125,	128,	30,	30,	30,	37,	9,	12,	100
48	72.6	52,	55,	63,	72,	33,	37,	39,	52,	11,	13,	120
51	68.4	75,	82,	85,	104,	29,	32,	35,	38,	15,	18,	100
54	60.9	81,	87,	89,	109,	41,	45,	58,	67,	13,	17,	85
57	66.4	94,	102,	105,	123,	43,	45,	49,	49,	12,	24,	70
60	83.8	69,	72,	86,	89,	38,	39,	57,	57,	13,	17,	100
63	69.5	77,	86,	89,	92,	35,	36,	36,	45,	9,	18,	100
66	63.5	69,	71,	71,	82,	36,	38,	50,	50,	11,	17,	100
69	67.4	52,	61,	64,	67,	42,	45,	49,	56,	9,	25,	90
72	53.4	77,	92,	99,	144,	49,	51,	57,	71,	17,	21,	70
75	61.2	74,	75,	77,	79,	46,	51,	52,	56,	25,	26,	80
81	84.3	90,	100,	112,	114,	43,	46,	46,	47,	13,	16,	70
87	54.4	96,	103,	103,	112,	33,	37,	50,	58,	19,	22,	80
92	62.8	59,	94,	110,	112,	40,	43,	46,	75,	18,	18,	90

Perfusate made up to the 250 ml. mark with 100 ppm. 2-CPA solution. Sampled after 1 hr. perfusion. Day 95.

95	69.6	76,	79,	94,	106,	30,	34,	40,	46,	13,	13,	120
100	69.6	86,	86,	88,	108,	25,	27,	32,	40,	10,	15,	130
110	77.6	67,	88,	92,	110,	39,	40,	40,	41,	17,	18,	105
130	69.3	87,	95,	107,	117,	45,	52,	53,	65,	30,	36,	40
135	79.1	85,	96,	104,	114,	44,	48,	49,	52,	18,	24,	55
140	62.5	88,	98,	107,	123,	37,	43,	47,	48,	13,	16,	80

Table 17. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
150,	54.4,	118,	118,	132,	146,	55,	57,	61,	83,	24,	40,	30,
160,	52.3,	79,	86,	88,	117,	31,	40,	46,	52,	17,	27,	70,
170,	46.3,	75,	84,	91,	110,	45,	54,	65,	69,	15,	24,	65,
180,	44.1,	98,	100,	111,	141,	52,	64,	68,	84,	39,	39,	20,
190,	46.3,	78,	84,	95,	106,	67,	69,	73,	91,	30,	43,	15,
200,	55.2,	58,	65,	82,	109,	69,	69,	71,	78,	34,	40,	25,
210,	50.9,	75,	75,	77,	102,	57,	61,	67,	83,	30,	31,	25,
220,	63.3,	84,	90,	95,	98,	43,	49,	65,	87,	30,	36,	35,
230,	59.4,	74,	76,	76,	86,	59,	59,	64,	101,	34,	47,	30,
240,	61.9,	86,	91,	91,	94,	42,	49,	50,	60,	24,	29,	50,
250,	62.8,	83,	88,	89,	94,	67,	67,	78,	81,	27,	27,	35,
260,	57.9,	90,	100,	112,	112,	47,	48,	57,	67,	24,	28,	40,
270,	49.2,	106,	108,	116,	126,	65,	65,	71,	75,	26,	31,	25,
280,	54.6,	75,	88,	92,	93,	37,	44,	44,	44,	11,	13,	80,
290,	67.4,	86,	88,	92,	98,	52,	54,	61,	62,	33,	34,	35,
300,	67.1,	97,	100,	103,	116,	58,	58,	61,	63,	15,	18,	30,
310,	76.6,	95,	98,	98,	100,	60,	61,	63,	64,	21,	21,	35,
320,	65.9,	94,	99,	100,	117,	55,	56,	56,	65,	40,	41,	35,
330,	69.8,	90,	93,	95,	107,	53,	58,	65,	70,	34,	40,	30,
340,	70.5,	98,	102,	105,	109,	71,	74,	75,	86,	28,	30,	20,
350,	45.1,	80,	84,	84,	95,	44,	47,	58,	62,	22,	27,	45,

Table 17a. Direct perfusion of 2-chlorophenoxyacetic acid.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.1 ppm. as percentage of mean control.
D. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
E. Longest roots at nominal concentration of 10 ppm. as % m.c.
F. Indicated 2-CPA concentration in the perfusate (ppm.).

Perfusion started on 20/12/51 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried June 1951,) and 250 ml. of 100 ppm. 2-CPA solution. First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	52.3	73,	78,	84,	88,	31,	34,	38,	44,	16,	18,	100
10	52.3	69,	74,	77,	86,	40,	42,	46,	48,	13,	17,	110
13	81.3	77,	84,	88,	101,	28,	34,	42,	49,	12,	16,	100
16	77.7	62,	95,	106,	106,	27,	31,	41,	49,	8,	11,	120
19	72.0	79,	81,	92,	92,	38,	39,	45,	46,	7,	9,	90
22	59.0	56,	73,	85,	107,	25,	31,	32,	37,	12,	17,	110
25	59.7	72,	84,	89,	97,	42,	47,	47,	49,	15,	17,	100
28	47.2	74,	79,	81,	93,	28,	30,	40,	43,	13,	17,	110
31	51.2	98,	104,	108,	127,	43,	43,	53,	55,	14,	16,	90
33	43.2	91,	91,	123,	123,	56,	63,	63,	72,	21,	28,	55
36	58.1	81,	83,	86,	86,	36,	38,	41,	43,	11,	12,	105
39	74.6	73,	73,	77,	103,	36,	37,	41,	49,	17,	19,	90
42	67.0	94,	96,	105,	111,	46,	48,	49,	55,	22,	24,	60
45	57.1	70,	74,	88,	90,	56,	56,	61,	67,	14,	21,	90
48	72.6	68,	73,	76,	101,	45,	48,	52,	69,	20,	30,	65
51	68.4	53,	58,	60,	82,	32,	48,	56,	76,	19,	25,	45
54	60.9	66,	74,	95,	112,	54,	69,	69,	74,	13,	15,	90
57	66.4	67,	78,	81,	88,	42,	48,	51,	55,	20,	20,	70
60	83.8	77,	78,	83,	106,	52,	56,	62,	62,	23,	28,	40
63	69.5	92,	97,	104,	105,	36,	46,	59,	69,	5,	10,	50
66	63.5	91,	95,	101,	103,	44,	49,	55,	68,	21,	22,	50
69	67.4	83,	91,	101,	110,	46,	48,	52,	64,	30,	37,	40
72	53.4	86,	99,	100,	103,	47,	50,	54,	60,	22,	30,	45
75	61.2	88,	92,	121,	134,	69,	71,	82,	92,	23,	26,	40
81	84.3	72,	82,	83,	100,	62,	66,	71,	75,	16,	27,	60
87	54.4	79,	81,	83,	90,	42,	48,	48,	55,	33,	44,	60
92	62.8	89,	91,	91,	120,	24,	35,	42,	43,	21,	26,	80

Perfusate made up to the 250 ml. mark with 100 ppm. 2-CPA solution. Sampled after 1 hr. perfusion. Day 95.

95	69.6	64,	80,	92,	96,	50,	50,	72,	73,	19,	24,	50
100	69.6	68,	68,	89,	111,	26,	35,	40,	48,	18,	19,	100
110	77.6	88,	94,	98,	99,	61,	62,	66,	80,	20,	28,	40
130	69.3	86,	87,	89,	97,	52,	59,	65,	82,	30,	35,	30
135	79.1	92,	96,	96,	104,	41,	44,	46,	48,	18,	22,	75
140	62.5	83,	85,	109,	111,	55,	61,	66,	72,	30,	35,	30
150	54.4	112,	116,	135,	140,	33,	42,	44,	74,	33,	37,	80

Table 17a. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
160,	52.3,	79,	88,	99,	107,	46,	55,	63,	71,	19,	21,	50,
170,	46.3,	58,	65,	82,	102,	50,	60,	69,	75,	41,	50,	25,
180,	44.1,	75,	91,	93,	111,	34,	36,	41,	45,	11,	18,	80,
190,	46.3,	86,	91,	97,	108,	30,	32,	39,	44,	19,	22,	50,
200,	55.2,	76,	83,	92,	98,	52,	56,	71,	83,	18,	24,	55,
210,	50.9,	94,	94,	100,	106,	75,	77,	79,	88,	28,	31,	25,

Perfusate made up to the 250 ml. mark with 100 ppm. 2-CPA solution. Sampled after 1 hr. perfusion. Day 220.

220,	63.3,	82,	82,	90,	97,	43,	47,	49,	51,	18,	19,	65,
230,	59.4,	88,	89,	96,	101,	49,	50,	62,	64,	24,	25,	40,
240,	61.9,	58,	62,	70,	74,	37,	42,	47,	49,	24,	26,	80,
250,	62.8,	84,	86,	88,	88,	37,	41,	43,	72,	15,	22,	80,
260,	57.9,	62,	67,	69,	76,	36,	38,	38,	40,	28,	29,	110,
270,	49.2,	77,	77,	81,	124,	37,	39,	41,	67,	31,	39,	100,
280,	54.6,	64,	68,	70,	92,	48,	53,	57,	64,	13,	19,	95,
290,	67.4,	94,	100,	106,	107,	52,	61,	62,	71,	21,	42,	25,
300,	67.1,	82,	85,	85,	88,	64,	66,	66,	75,	27,	33,	25,
310,	76.6,	95,	97,	107,	107,	60,	63,	68,	78,	27,	27,	30,
320,	65.9,	76,	85,	86,	93,	53,	56,	58,	70,	20,	21,	50,
330,	69.8,	69,	70,	80,	82,	40,	40,	42,	43,	33,	40,	95,
340,	70.5,	96,	98,	116,	118,	74,	75,	77,	94,	21,	34,	30,
350,	45.1,	95,	98,	107,	122,	58,	69,	69,	78,	27,	27,	30,

Table 17b. Direct perfusion of 2-chlorophenoxyacetic acid.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.1 ppm. as percentage of mean control.
D. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
E. Longest roots at nominal concentration of 10 ppm. as % m.c.
F. Indicated 2-CPA concentration in the perfusate (ppm.).

Perfusion started on 13/5/52 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried April 1952,) and 250 ml. of 100 ppm. 2-CPA solution. First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	69.0	70,	74,	76,	109,	33,	36,	44,	51,	13,	22,	90
5	54.4	85,	87,	96,	120,	37,	42,	46,	48,	17,	20,	85
15	52.3	81,	92,	96,	117,	38,	42,	63,	71,	14,	25,	70
25	46.3	95,	95,	130,	130,	39,	50,	56,	65,	7,	13,	45
35	44.1	80,	86,	98,	114,	41,	50,	57,	66,	16,	25,	50
45	46.3	84,	91,	99,	99,	48,	56,	63,	78,	11,	13,	40
55	55.2	63,	69,	100,	102,	36,	43,	72,	81,	18,	24,	75
65	50.9	79,	82,	88,	96,	41,	45,	49,	65,	14,	16,	80
75	63.3	78,	87,	87,	93,	35,	36,	36,	40,	10,	13,	80
85	59.4	81,	84,	89,	94,	44,	44,	47,	50,	15,	17,	100
95	61.9	74,	81,	81,	92,	36,	40,	40,	42,	18,	24,	100
105	62.8	73,	80,	83,	88,	37,	40,	54,	59,	15,	19,	90
115	57.9	67,	67,	76,	78,	29,	40,	40,	42,	9,	12,	110
125	49.2	77,	83,	83,	86,	43,	47,	47,	51,	14,	17,	90

Key to further columns in table:
A, B, D, E and F as above.
G. Longest roots at nominal concentration of 100 ppm. as % m.c.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	G.	G.	F.
135	54.6	55,	59,	64,	66,	22,	26,	29,	29,	3.5,	3.5,	30
145	67.4	42,	43,	45,	48,	21,	21,	22,	22,	4.5,	6.0,	70
155	67.1	55,	58,	58,	61,	24,	25,	36,	37,	7.5,	9.0	35
165	76.6	42,	48,	48,	63,	22,	24,	26,	29,	2.5,	2.5,	50
175	65.9	50,	50,	52,	53,	20,	24,	24,	29,	3.0,	4.5,	50
185	69.8	47,	49,	53,	60,	16,	16,	16,	21,	5.5,	7.0,	60
195	70.5	45,	50,	51,	69,	28,	30,	33,	40,	3.0,	4.5,	40
205	45.1	51,	55,	62,	62,	20,	24,	33,	38,	4.5,	4.5,	25

Table 18. Direct perfusion of 3-chlorophenoxyacetic acid.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.1 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
E. Longest roots at nominal concentration of 10 ppm. as % m.c.
F. Indicated 3-CPA concentration in the perfusate (ppm.).

Perfusion started on 24/6/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 10 ppm. 3-CPA solution. First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	98.1	80,	81,	84,	88,	43,	44,	46,	49,	20,	20,	10.0
10	99.3	79,	82,	92,	102,	35,	42,	45,	49,	16,	19,	10.5
15	95.2	74,	74,	77,	81,	39,	40,	41,	42,	22,	24,	14.0
20	97.3	78,	80,	84,	84,	43,	43,	48,	48,	19,	19,	11.0
25	105.1	87,	93,	97,	100,	47,	48,	49,	49,	19,	20,	9.0
30	102.6	89,	92,	93,	109,	45,	46,	49,	50,	21,	22,	10.0
40	97.1	80,	83,	85,	97,	61,	66,	66,	66,	25,	28,	5.5
50	97.5	101,	102,	102,	104,	70,	71,	77,	79,	37,	38,	2.0
55	101.4	92,	93,	101,	107,	69,	70,	73,	81,	32,	35,	2.0

Perfuser drained and refilled with 250 ml. of 10 ppm. 3-CPA solution. Perfusion restarted, sampled after 1 hr. Day 55.

55a	96.3	74,	80,	80,	81,	39,	39,	40,	40,	24,	25,	12.5
60	94.0	83,	84,	84,	85,	43,	44,	46,	52,	21,	22,	10.0
70	97.3	82,	86,	87,	91,	49,	50,	50,	51,	23,	27,	7.0

Table 18a. Direct perfusion of 3-chlorophenoxyacetic acid.

Key to columns, and details of perfuser set up, as in Table 18, above, except that the initial 3-CPA concentration was 100 ppm.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	98.1	78,	79,	83,	85,	41,	41,	43,	46,	17,	20,	110
10	99.3	86,	86,	91,	92,	48,	48,	54,	54,	15,	18,	80
15	95.2	79,	81,	89,	90,	43,	45,	45,	52,	19,	21,	105
20	97.3	82,	84,	88,	98,	42,	44,	48,	51,	24,	29,	100
25	105.1	86,	87,	101,	112,	48,	49,	50,	50,	21,	23,	90
30	102.6	84,	85,	88,	91,	52,	54,	56,	58,	20,	27,	90
40	97.1	80,	81,	82,	89,	46,	49,	53,	56,	23,	25,	80
50	97.5	79,	82,	83,	87,	43,	44,	47,	49,	22,	27,	100
55	101.4	84,	85,	93,	95,	42,	44,	51,	53,	22,	24,	90
60	94.0	83,	83,	86,	89,	49,	53,	54,	64,	21,	27,	80
70	97.3	88,	93,	95,	103,	46,	47,	49,	54,	24,	25,	75

Table 19. Perfusion of 4-CPA over crushed sterile pot; (attempted direct, and transferred, adaptation.).

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage of mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Indicated 4-CPA concentration in the perfusate (ppm.).

Perfusion started on 17/6/52 with 50 gm. of sterilised, washed 2 to 4 mm., crushed flower-pot and 250 ml. of 100 ppm. 4-CPA solution. This solution was made up in the filtrate from a 10 day old suspension of 1 Kg. of soil (fines, Sussex Lodge soil, dried February 1952,) in 2 L. of distilled water. First sample taken after 1.hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	44.1	77,	91,	109,	127,	25,	25,	25,	27,	4.5,	7,	100
3	45.2	66,	73,	111,	126,	24,	24,	29,	42,	4.5,	6.5,	90
6	47.0	81,	89,	94,	98,	26,	28,	30,	30,	4,	4,	80
9	59.4	62,	69,	109,	119,	30,	35,	35,	35,	5,	6.5,	55
12	48.3	91,	95,	97,	108,	29,	29,	44,	46,	4,	6,	70
15	41.9	93,	95,	122,	124,	29,	29,	31,	31,	7,	7,	80
18	47.0	87,	89,	106,	108,	28,	28,	30,	34,	6.5,	8.5,	80
21	43.4	74,	88,	92,	104,	42,	42,	42,	44,	9,	19,	45
24	50.5	93,	93,	99,	99,	20,	20,	26,	26,	2,	6,	105
27	48.7	80,	82,	86,	109,	23,	27,	33,	33,	4,	6,	80
30	50.9	73,	77,	94,	110,	33,	33,	37,	37,	6,	8,	60
33	44.3	61,	84,	102,	117,	23,	27,	32,	36,	4.5,	7,	80
36	47.3	68,	85,	87,	91,	30,	30,	30,	57,	6.5,	6.5,	80
51	59.4	66,	69,	69,	91,	22,	22,	24,	37,	5,	6.5,	120
54	50.9	75,	81,	81,	98,	24,	26,	26,	28,	4,	4,	100
57	55.4	74,	80,	85,	88,	22,	22,	23,	25,	3.5,	5.5,	110
60	61.9	53,	53,	60,	60,	20,	21,	21,	26,	3,	5,	130
63	65.8	55,	64,	64,	72,	21,	21,	26,	35,	3,	6,	110
66	49.6	50,	57,	75,	85,	20,	22,	24,	24,	4,	4,	110
69	66.9	75,	78,	103,	105,	20,	21,	22,	37,	4.5,	4.5,	100
72	86.2	88,	99,	102,	107,	18,	18,	18,	22,	2.5,	3.5,	120

Perfusate drained and made up to 250 ml. of 100 ppm. with "concentrate" (1% 4-CPA solution,) and "active" perfusate drained from another perfuser on the same day (Graph 21, 28/8/52,). Perfusion re-started, first sample taken after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
72a	43.4	67,	74,	76,	78,	28,	28,	30,	37,	4.5,	7,	90
75	68.8	74,	77,	81,	92,	25,	25,	26,	31,	6,	6,	100

Table 19. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
78,	72.0,	70,	76,	97,	100,	22,	28,	32,	42,	3,	4,	80,
81,	63.0,	99,	99,	99,	108,	52,	52,	62,	67,	6.5,	9.5,	30,
84,	71.8,	79,	81,	82,	97,	68,	82,	86,	86,	47,	53,	5,
87,	26.4,	68,	80,	83,	91,	91,	95,	95,	99,	64,	72,	1,

Approximately 2.5 ml. of 1% 4-CPA solution added to the perfuser, without draining, and water to make 250 ml. Perfusion re-started, sampled after 1 hr. Day 87.

87a,	26.4,	99,	102,	110,	110,	38,	38,	38,	45,	7.5,	7.5,	50,
90,	49.2,	77,	83,	92,	98,	39,	39,	41,	49,	8,	10,	45,
93,	56.9,	95,	100,	102,	104,	107,	111,	114,	118,	107,	127,	<1,

Approximately 2.5 ml. of 1% 4-CPA solution added to the perfuser, without draining, and water to make 250 ml. Perfusion re-started, sampled after 1 hr. Day 95.

95a,	61.4,	60,	68,	77,	85,	23,	23,	24,	33,	5,	5,	105,
102,	56.7,	92,	95,	97,	101,	70,	83,	83,	95,	78,	90,	<1,
104,	74.3,	97,	101,	101,	107,	100,	107,	119,	124,	97,	105,	<1,
106,	56.7,	74,	74,	83,	95,	56,	56,	67,	76,	65,	88,	<1,

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA. Perfusion re-started, sampled after 1 hr. Day 106.

106a,	74.3,	74,	77,	78,	86,	24,	27,	28,	34,	5.5,	6.5,	95,
108,	74.3,	70,	73,	75,	81,	24,	26,	35,	39,	6.5,	8,	80,
110,	74.3,	96,	97,	103,	103,	94,	96,	96,	108,	82,	90,	<1,
112,	74.3,	80,	88,	89,	93,	86,	88,	88,	98,	82,	96,	<1,
114,	75.3,	81,	86,	89,	93,	86,	89,	89,	96,	70,	92,	<1,

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA. Perfusion re-started, sampled after 1 hr. Day 114.

114a,	75.3,	64,	67,	68,	80,	45,	47,	48,	49,	5.5,	8,	60,
117,	61.6,	83,	91,	91,	93,	76,	86,	93,	104,	81,	81,	<1,
120,	75.3,	83,	88,	94,	105,	75,	81,	81,	83,	100,	107,	<1,

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA. Perfusion restarted, sampled after 1 hr. Day 120.

120a,	75.3,	56,	59,	64,	85,	24,	24,	33,	33,	4,	4,	100,
123,	81.3,	86,	92,	96	107,	81,	90,	95,	95,	74,	74,	1,
126,	78.3,	104,	105,	107,	107,	86,	93,	97,	105,	101,	107,	<1,
129,	69.6,	105,	105,	111,	115,	83,	88,	98,	103,	78,	95,	<1,

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA. Perfusion restarted, sampled after 1 hr. Day 129.

129a,	73.7,	54,	56,	68,	76,	22,	24,	24,	27,	5.5,	9.5,	100,
131,	72.5,	94,	97,	113,	117,	87,	91,	92,	92,	97,	101,	<1,

Table 19. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
133,	73.7,	90,	98,	102,	102,	80,	85,	92,	94,	84,	90,	<1,

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA. Perfusion restarted, sampled after 1 hr. Day 133.

133a,	73.7,	73,	73,	75,	77,	24,	24,	26,	35,	4,	7,	100,
135,	60.3,	108,	111,	120,	123,	88,	90,	105,	118,	76,	85,	1,
137,	72.2,	97,	98,	101,	107,	87,	87,	88,	93,	101,	122,	<1,

Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 4-CPA and 0.1% sodium fluoride. Perfuser restarted, sampled after 1 hr. Samples taken daily from this point and tested for "phenols". Day 137.

137a,	72.2,	82,	83,	88,	88,	25,	26,	32,	32,	5.5,	5.5,	80,
139,	65.2,	104,	113,	115,	126,	77,	81,	83,	83,	71,	74,	1,
141,	66.0,	91,	95,	101,	106,	98,	103,	108,	112,	98,	104,	<1,

Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 4-CPA and 0.5% sodium fluoride. Perfuser restarted, sampled after 1 hr. Day 143.

143a,	57.7,	80,	81,	81,	83,	28,	29,	29,	43,	7,	8.5,	80,
145,	65.0,	109,	111,	117,	119,	98,	103,	108,	119,	106,	123,	<1,
147,	68.8,	96,	108,	119,	121,	93,	95,	99,	102,	93,	102,	<1,

Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 4-CPA and 1.0% sodium fluoride. Perfuser restarted, sampled after 1 hr. Day 147.

147a,	68.8,	87,	95,	99,	105,	32,	36,	38,	45,	4.5,	7.5,	50,
149,	66.7,	72,	72,	75,	78,	53,	62,	69,	74,	4.5,	7.5,	60,
151,	74.4,	73,	77,	90,	93,	30,	34,	36,	43,	6.5,	8,	55,

Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 4-CPA and 5% sodium fluoride. Perfuser restarted, sampled after 1 hr. Day 151.

151a,	59.7,	70,	75,	75,	87,	22,	23,	25,	28,	0,	1.5,	100,
153,	73.1,	75,	85,	86,	94,	22,	26,	29,	30,	0,	0,	80,
155,	81.2,	76,	78,	79,	79,	27,	31,	33,	36,	0,	1,	70,
157,	66.5,	117,	119,	119,	120,	21,	24,	33,	39,	0,	4.5,	80,
159,	76.2,	72,	73,	79,	83,	26,	26,	28,	37,	0,	0,	90,
161,	79.2,	76,	81,	86,	87,	23,	24,	28,	37,	0,	0,	80,
163,	77.9,	87,	92,	99,	99,	23,	24,	24,	27,	0,	0,	105,
165,	66.0,	42,	50,	52,	52,	20,	21,	26,	27,	0,	3,	110,

Table 19. continued.

Concentration of phenolic compounds (Folin and Ciocalteu) in the perfusate during fluoride poisoning of 4-CPA breakdown. Estimated as 4-chlorphenol.

Key to columns in table:
A. Day of perfusion.
B. Colourimeter reading (E.E.L.) in divisions.
C. Indicated "4-CP" concentration in the perfusate (ppm.).

A.	B.	C.	
137,	1.0,	1.3,	
138,	1.2,	1.6,	
139,	1.8,	2.4,	
140,	2.2,	2.9,	
141,	2.5,	3.3,	
142,	2.2,	2.9,	
143,	1.4,	1.9,	Perfusate before draining.
143,b,	0.0,	0.0,	4-CPA solution before adding to perfuser.
143,a,	0.0,	0.0,	Perfusate after 1 hr.
144,	0.4,	0.5,	
145,	1.1,	1.5,	
146,	1.3,	1.7,	
147,	2.0,	2.7,	Perfusate before draining.
147,b,	0.0,	0.0,	4-CPA solution before adding to perfuser.
147,a,	0.0,	0.0,	Perfusate after 1 hr.
148,	0.0,	0.0,	
149,	1.0,	1.3,	
150,	0.8,	1.1,	
151,	0.9,	1.2,	Perfusate before draining.
151,b,	1.8,	2.4,	4-CPA solution before adding to perfuser.
151,a,	3.0,	4.0,	Perfusate after 1 hr.
152,	4.0,	5.3,	
153,	2.1,	2.8,	
154,	4.5,	6.0	
155,	3.2,	4.3,	
156,	3.4,	4.5,	
157,	5.0,	6.7,	
158,	3.7,	4.9,	
159,	3.8,	5.1,	
160,	4.1,	5.5,	
161,	5.0,	6.7,	
162,	5.1,	6.8,	
163,	4.9,	6.5,	

Table 20. Perfusion of 4-CPA over crushed, sterile pot; (attempted direct, and transferred, adaptation.).

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Indicated 4-CPA concentration in the perfusate (ppm.).
G. Colourimeter reading (Divs. on the E.E.L. instrument.).
H. Indicated 4-chlorphenol concentration in the perfusate(ppm).
I. Indicated 2,4-D concentration in the suspension (ppm.).

Perfusion started on 17/6/52 with 50 gm. of sterilised, washed, 2 to 4 mm., crushed flower pot and 250 ml. of 100 ppm. 4-CPA solution. This solution was made up in the filtrate from a 10 day old suspension of 1 Kg. of soil (fines, Sussex Lodge soil, dried February 1952,) in 2 L. of distilled water. First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	44.1	77,	91,	93,	104,	34,	36,	39,	41,	7,	9,	55
3	45.2	80,	82,	115,	115,	29,	31,	33,	44,	7,	9,	65
6	47.0	102,	104,	106,	128,	23,	26,	26,	32,	4,	7,	100
9	59.4	92,	94,	99,	101,	27,	27,	32,	32,	7,	9,	65
12	48.3	83,	95,	101,	120,	29,	31,	33,	37,	4,	6,	65
15	41.9	95,	103,	112,	127,	31,	31,	33,	43,	7,	7,	65
18	47.0	87,	102,	106,	134,	26,	32,	34,	43,	4,	7,	65
21	43.4	81,	92,	108,	111,	30,	32,	39,	41,	7,	9,	60
24	50.5	63,	79,	97,	103,	24,	30,	32,	32,	4,	6,	55
27	48.7	86,	88,	93,	97,	25,	27,	29,	33,	4,	6,	80
30	50.9	94,	98,	98,	122,	22,	24,	28,	30,	4,	6,	80
33	44.3	56,	63,	70,	82,	29,	29,	32,	34,	5,	5,	80
36	47.3	72,	76,	87,	102,	23,	25,	36,	38,	4,	4,	75
51	59.4	91,	93,	98,	109,	20,	24,	24,	30,	4,	5,	80
54	50.9	69,	75,	88,	96,	24,	24,	24,	43,	4,	4,	100
57	55.4	72,	76,	76,	87,	25,	27,	29,	33,	4,	6,	90
60	61.9	49,	66,	71,	74,	24,	24,	29,	31,	3,	5,	100
63	65.8	55,	61,	68,	81,	15,	17,	17,	18,	3,	3,	160
66	49.6	57,	57,	67,	71,	16,	16,	18,	22,	4,	4,	170
69	66.9	72,	84,	85,	88,	15,	17,	22,	31,	3,	3,	125
72	86.2	62,	65,	66,	77,	13,	14,	15,	21,	2.5,	2.5,	175
75	68.8	31,	32,	36,	49,	16,	16,	22,	22,	3,	3,	160
78	72.0	43,	46,	53,	57,	11,	12,	13,	20,	1.5,	3,	290
81	63.0	46,	48,	48,	56,	9,	11,	11,	14,	1.5,	3,	315

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution prepared from the"active" perfusate drained from another perfuser on the same day (Graph 21, 9/9/52.). Perfusion re-started, first sample taken after 1 hr. Day 84.

Table 20. continued.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
84a	56.7	49, 53, 78,108,	25, 26, 26, 26,	3.5,5.5,	100
87	26.4	91, 99,121,121,	45, 45, 49, 53,	7.5,7.5,	35
90	49.2	65, 65, 67, 77,	26, 28, 33, 33,	6, 8,	90
93	56.9	67, 76, 81,100,	23, 25, 25, 26,	5.5, 7,	100
96	53.3	87, 90, 90,103,	32, 34, 38, 38,	7.5,7.5,	55
102	63.5	66, 68, 69, 83,	80, 85, 91, 95,	66, 77,	5
105b	63.5	95, 98,112,120,	84,105,107,113,	80, 93,	<1

Perfusate made up to 250 ml. of approximately 100 ppm. 4-CPA without draining. Perfusion re-started, sampled after 1 hr. perfusion. Day 105.

105a	63.5	76, 84, 91,110,	33, 39, 43, 44,	4.5,6.5,	65
108	67.6	67, 75, 78, 84,	34, 34, 36, 37,	6,7.5,	60
111	55.1	89,100,112,116,	34, 45, 47, 51,	12, 14,	30
114b	75.3	85, 87, 93,111,	93, 93,106,109,	89, 92,	<1

Perfusate made up to 250 ml. of 100 ppm. 4-CPA by adding water and 4-CPA "concentrate". Perfusion restarted, sampled after 1 hr.

114a	75.3	59, 62, 63, 83,	26, 26, 28, 30,	4,6.5,	100
117	61.6	60, 63, 76, 83,	21, 23, 23, 29,	6.5, 8,	110
120	67.1	89,100,116,122,	91, 94,101,116,	86, 97,	<1
123b	80.3	94, 94, 95,101,	75, 81, 81,118,	77,107,	<1

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA. Perfusion re-started, sampled after 1 hr.

123a	80.3	77, 78, 86,103,	29, 29, 31, 31,	5, 5,	95
126	80.3	76, 96,107,110,	91, 91, 95,106,	77,106,	<1
129b	69.6	98,101,106,108,	95, 99,105,108,	73, 83,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA. Perfusion re-started, sampled after 1 hr.

129a	73.7	68, 69, 72, 75,	19, 19, 22, 30,	2.5, 4,	100
131	72.5	80, 98,103,110,	102,104,110,124,	91,113,	<1
133b	73.7	86, 86, 90, 92,	87, 95, 99,106,	100,100,	<1

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfusion re-started, sampled after 1 hr.

133a	73.7	73, 77, 79, 90,	26, 27, 30, 41,	5.5,5.5,	95
135	60.3	109,111,113,115,	91, 91, 98,105,	88,105,	<1
137b	72.2	91,100,102,104,	108,108,111,125,	107,113,	<1

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfusion restarted, sampled after 1 hr.

137a	72.2	65, 71, 73, 75,	25, 25, 26, 26,	4, 4,	105
139	65.2	103,104,115,120,	115,120,126,129,	94,120,	<1
141b	66.0	100,100,118,118,	86, 89, 89, 95,	77, 99,	<1

Table 20. continued.

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfusion restarted, sampled after 1 hr. Day 141.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
141a	66.0	48,	50,	53,	80,	23,	24,	30,	33,	6,	7.5,	90
143	57.7	81,	100,	102,	107,	85,	90,	106,	113,	85,	90,	1
145	65.0	103,	120,	121,	128,	92,	94,	98,	119,	119,	135,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-chlorphenol solution. Solution sampled before adding to the perfuser. Perfusion re-started, sampled after 1 hr. Day 145.

A.	G.	H.	A.	G.	H.	A.	G.	H.
145ba	81	108	148	61	81	152	38.2	51
145a	72.5	96	149	57.3	76	153	30.2	40
146	66	88	150	54.2	72	154b	3.1	4
147	64.5	86	151	43.5	58			

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CP solution. Solution sampled before adding to the perfuser. Perfusion restarted, sampled after 1 hr. Day 154.

154ba	77	103	156	58	77	159	28	37
154a	69.5	93	157	52	69	160b	2.8	4
155	66	88	158	42.3	56			

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CP solution. Solution sampled before adding to the perfuser. Perfusion re-started, sampled after 1 hr. Day 160.

160ba	76	101	163	62.5	84	167	32.5	44
160a	70	93	164	55.5	74	168	18	24
161	68	91	165	51	68	169b	1.7	2
162	66	88	166	46	61			

25 ml. of 1,000 ppm. 4-CP solution added to the perfuser without draining, making the perfusate approximately 250 ml. of 100 ppm. Sampled after 1 hr. perfusion. Day 169.

169a	71	95	174	57	76	179	24.4	32
170	64.7	86	175	46	61	180	21	28
171	64.2	86	176	41	55	181b	9	12
172	66	88	177	38	51			
173	58	77	178	36	48			

25 ml. of 1,000 ppm. 4-CP solution added to the perfuser without draining, making the perfusate approximately 250 ml. of 100 ppm. Sampled after 1 hr. perfusion. Day 181.

181a	74	99	183	68.5	91	205b	0	0
182	72	96						

Table 20. continued.

25 ml. of 1,000 ppm. 4-CP solution added to the perfuser without draining, making the perfusate approximately 250 ml. of 100 ppm. Sampled after 1 hr. perfusion. Day 205.

A.	G.	H.	A.	G.	H.	A.	G.	H.
205a	76.5	102	209	61.5	82	213	33	44
206	69.5	93	210	60	80	214	19	25
207	69.5	93	211	54	72	215b	2.8	4
208	68	91	212	38.5	52			

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CP solution. Solution sampled before adding, perfusion re-started, sampled after 1 hr. perfusion. Day 215.

215ba	78.5	104	219	60	80	224	37	49
215a	74	99	220	56.5	75	225	30.5	40
216	70	93	221	51.0	68	226	20.5	27
217	66.5	89	222	43	57	227	11	15
218	66	88	223	44.5	59	228b	2.5	3

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CP solution. Solution sampled before adding, perfusion re-started, sampled after 1 hr. perfusion. Day 228.

228ba	76.5	102	232	50	67	237	26	37
228a	66.5	89	233	44.5	59	238	18	24
229	61.5	82	234	41.5	55	239	12	16
230	58	77	235	34.2	45	240b	7.1	9
231	53.5	71	236	31.0	41			

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CP solution. Solution sampled before adding, perfusion re-started, sampled after 1 hr. perfusion. Day 240.

240ba	79	105	243	56	75	247	21	28
240a	67	89	244	51	68	248b	9.5	13
241	62.5	83	245	43	57			
242	59	79	246	33	44			

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CP solution. Solution sampled before adding, perfusion re-started, sampled after 1 hr. perfusion. Day 248.

248ba	78	104	251	44.5	59	255	20	27
248a	68.5	92	252	37.5	50	256	15	20
249	63	84	253	31.5	42	257	9.5	12
250	53	71	254	26	35	258b	7.6	10

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfusion re-started, sampled after 1 hr. Day 258.

Table 20. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
258	85.3	70,	79,	80,	80,	21,	21,	26,	29,	4.5,	4.5,	100
260	86.1	70,	78,	78,	81,	20,	23,	24,	29,	4.5,	7,	100
262	75.9	83,	92,	93,	99,	33,	34,	42,	45,	6.5,	8,	55
264	76.0	70,	76,	87,	91,	37,	37,	38,	38,	11,	13,	50
266	91.3	88,	96,	96,	109,	80,	81,	86,	96,	62,	75,	1
268	88.4	68,	72,	80,	86,	81,	88,	90,	95,	85,	88,	<1
270b	84.1	100,	100,	111,	115,	77,	80,	81,	84,	61,	64,	2

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfusion re-started, sampled after 1 hr. Day 270.

270a	78.7	72,	79,	80,	113,	23,	24,	24,	28,	4,	5,	100
272	71.0	73,	73,	74,	83,	37,	41,	52,	52,	16,	17,	30
274	82.7	97,	99,	101,	107,	106,	107,	118,	119,	92,	95,	<1

Perfusate and crushed pot from the column shaken together in a sterile flask, allowed to settle and the supernatant liquid decanted off into a further sterile flask. Made up to 250 ml. of 100 ppm.4-CPA with distilled water and 4-CPA "concentrate". Suspension well shaken and sampled. Day 277.

277a	72.5	43,	44,	52,	55,	16,	16,	17,	18,	5.5,	7,	200
283	77.8	53,	58,	72,	74,	15,	18,	19,	19,	3,	4,	160
286	77.1	58,	65,	71,	82,	25,	26,	29,	34,	8,	8,	100
289	85.2	70,	71,	82,	89,	27,	28,	30,	34,	8,	11,	80
295	81.9	83,	83,	84,	85,	70,	76,	76,	85,	35,	37,	10
301	80.0	61,	63,	69,	91,	86,	90,	95,	98,	86,	105,	<1
304b	81.7	86,	92,	95,	98,	95,	97,	98,	103,	95,	99,	<1

2.5 ml. of 1% 4-CPA solution added to make the suspension up to approximately 250 ml. of 100 ppm. Suspension incubated, sampled after 1 hr. Day 304.

304a	81.7	66,	68,	74,	94,	17,	17,	21,	21,	3.5,	5,	160
307	73.9	65,	69,	76,	78,	22,	23,	24,	28,	5.5,	5.5,	100
310	76.7	82,	85,	86,	88,	21,	22,	29,	31,	6.5,	6.5,	70
313	82.0	85,	88,	89,	105,	74,	74,	74,	77,	12,	19,	20
316	81.9	99,	104,	104,	110,	99,	100,	105,	107,	120,	122,	<1

2.5 ml. of 1% 4-CPA solution added to make the suspension up to approximately 250 ml. of 100 ppm. Suspension incubated at 28°C, sampled after 1 hr. Day 319.

319a	75.5	68,	74,	84,	94,	20,	24,	26,	29,	5.5,	5.5,	100
322	83.9	76,	77,	93,	105,	19,	19,	21,	24,	5,	6,	100
325	80.6	87,	92,	97,	100,	31,	32,	36,	41,	5,	7.5,	55
328	88.6	106,	109,	113,	114,	95,	96,	97,	101,	42,	44,	4
331	75.1	118,	120,	120,	126,	105,	121,	124,	124,	120,	122,	<1
334b	80.3	104,	112,	112,	113,	109,	109,	119,	123,	113,	124,	<1

Table 20. continued.

2.5 ml. of 1% 4-CPA solution added to make the suspension up to approximately 250 ml. of 100 ppm. Suspension incubated at 28°C, sampled after shaking and allowing to settle. Day 334.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
334a	80.3	82, 84, 90, 94,	20, 24, 24, 27,	5, 6,	90
337	90.0	87, 94, 97,100,	28, 30, 30, 33,	8, 11,	75
340	88.3	90, 90, 92, 96,	83, 85, 86, 87,	17, 19,	15
343	92.6	99,101,105,111,	99,104,105,112,	89, 89,	1

2.5 ml. of 1% 4-CPA solution added to make the suspension up to approximately 250 ml. of 100 ppm. Suspension incubated at 28°C, sampled after shaking and allowing to settle. Day 346.

346a	93.3	87, 87, 88, 89,	20, 21, 22, 22,	3,4.5,	125
349	93.9	89, 90, 93,102,	33, 37, 38, 39,	5.5,6.5,	50
352	92.3	94, 94,101,104,	43, 43, 51, 58,	7.5, 10,	40
355	98.6	99,104,105,112,	94, 96, 99,105,	15, 22,	15
358	95.2	95, 96, 98,108,	98,100,101,104,	100,101,	1

50 ml. of clear liquid pipetted off from above the soil in the settled suspension and used to start another suspension (Table 20a,). 2.5 ml. of 1% 4-CPA solution added to the residue along with sufficient water to give 250 ml. of 100 ppm. Incubated at 28°C, sampled after shaking and allowing to settle. Day 361.

361a	94.9	69, 73, 77, 77,	21, 22, 23, 28,	4, 4,	105
364	94.9	87, 87, 90,104,	35, 39, 41, 47,	6.5,7.5,	55
367	94.4	97, 98,101,104,	69, 72, 79, 95,	14, 15,	20
370	101.7	87, 88, 91, 96,	94,102,107,108,	63, 66,	5
373b	98.1	96, 96,103,109,	94, 94,105,117,	107,111,	1

2.5 ml. of 1% 4-CPA solution added to make the suspension up to approximately 250 ml. of 100 ppm. Suspension incubated at 28°C, sampled after shaking and allowing to settle. Day 373.

373a	100.1	95, 96, 97,109,	33, 33, 33, 37,	4, 5,	65
376	102.9	86, 89, 90, 92,	39, 41, 44, 52,	7, 8,	55
379	101.4	91, 93, 95,102,	57, 57, 63, 78,	10, 12,	25
382	99.3	93, 96,103,104,	94, 94,105,109,	97,109,	1

2.5 ml. of 1% 4-CPA solution added to make the solution up to approximately 250 ml. of 100 ppm. Suspension incubated at 28°C, sampled after shaking and allowing to settle. Day 385.

385a	105.4	88, 90, 92,101,	27, 30, 31, 33,	4,4.5,	65
388	99.9	87, 92, 98,101,	45, 49, 55, 58,	7, 8,	45
391	102.9	92, 92, 93,105,	69, 70, 75, 79,	21, 22,	12
394	99.6	90, 95, 96,109,	92, 93,105,118,	75, 75,	1

Table 20. continued.

2.5 ml. of 1% 2,4-D added to make the solution up to approximately 250 ml. of 100 ppm. Suspension incubated at 28°C, sampled after shaking and allowing to settle. Day 397.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	I.
397a	105.1	87,	88,	90,	93,	23,	24,	24,	30,	5,	7.5,	100
400	102.9	86,	88,	94,	96,	20,	22,	23,	26,	2,	3,	100

A further 2.5 ml. of 1% 2,4-D solution added by mistake, making the suspension approximately 250 ml. of 200 ppm. Suspension incubated at 28°C, sampled after shaking and allowing to settle. Day 403.

403a	100.1	70,	72,	73,	82,	12,	13,	17,	17,	2,	2,	185
406	101.2	70,	75,	77,	83,	12,	15,	15,	16,	2,	3,	200
409	96.3	69,	72,	77,	87,	11,	12,	12,	13,	2,	2,	200
412	99.4	65,	71,	71,	78,	13,	13,	13,	17,	4,	5,	200
415	100.0	65,	73,	73,	75,	9,	9,	10,	13,	2,	3,	205
418	93.9	74,	75,	82,	83,	10,	11,	12,	15,	2,	3,	200
427	101.4	61,	62,	71,	78,	13,	13,	14,	14,	2,	2,	200
439	97.0	71,	73,	76,	76,	13,	14,	15,	16,	3,	3,	180

Table 20a. Breakdown of 4-CPA in soil-free suspension, followed by 2,4-D.

Key to columns in table:
- A. Day of perfusion.
- B. Mean control root length (mm.).
- C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
- D. Longest roots at nominal dilution concentration of 0.1 ppm. as percentage mean control.
- E. Longest roots at nominal dilution concentration of 1.0 ppm. as percentage mean control.
- F. Indicated 4-CPA concentration in the suspension (ppm.).
- G. Indicated 2,4-D concentration in the suspension (ppm.).

Suspension started on 13/6/53. 200 ml. of distilled water was boiled gently with 2.5 ml. of 1% 4-CPA solution for 15 mins. After cooling, 50 ml. of the clear liquid, pipetted off from a settled soil suspension (Table 20, Day 361,), was added to the solution making it 100 ppm. with regard to 4-CPA. After shaking and sampling, the flask was plugged and incubated at 28°c.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
0	98.1	91, 93, 99,107,	27, 30, 30, 33,	4, 5,	80
10	98.1	101,102,111,113,	103,103,104,105,	94,107,	<1
15b	102.9	93, 94, 97,104,	86, 88, 93,107,	76, 84,	<1

Day 15. 2.5 ml. of 1% 4-CPA solution added to the suspension making it approximately 250 ml. of 100 ppm. 4-CPA. Suspension well shaken and sampled.

15a	102.9	78, 79, 80, 86,	26, 26, 28, 31,	3, 4,	90
20	97.5	83, 85, 87, 88,	102,105,106,108,	99,100,	<1
23b	98.6	89, 92, 97,100,	101,103,103,113,	101,103,	<1

Day 23. 2.5 ml. of 1% 4-CPA solution added to the suspension making it approximately 250 ml. of approximately 100 ppm. 4-CPA. Suspension well shaken and sampled.

23a	98.6	85, 90, 98, 99,	26, 28, 30, 31,	3, 4,	85
26	95.2	98, 99,105,116,	93,103,103,112,	94,100,	<1

Day 29. 2.5 ml. of 1% 4-CPA solution added to the suspension making it approximately 250 ml. of 100 ppm. 4-CPA. Suspension well shaken and sampled.

29a	95.3	81, 87, 92,107,	30, 31, 32, 36,	3,6.5,	70
31	97.3	99,100,104,110,	46, 50, 54, 66,	9.5, 12,	30
33	99.6	106,107,108,111,	88, 90, 93, 99,	30, 33,	10
35b	98.4	96, 96, 97,100,	96,101,101,104,	92, 93,	<1

Table 20a. continued.

Day 35. 2.5 ml. of 1% 4-CPA solution added to the suspension making it approximately 250 ml. of 100 ppm. 4-CPA. Suspension well shaken and sampled.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
35a	95.9	96,100,104,104,	38, 39, 43, 45,	4, 4,	40
38	102.9	95, 97,100,105,	44, 46, 47, 50,	8,8.5,	30
41	102.6	92,101,101,108,	105,107,108,108,	75, 80,	<1

Day 44. 2.5 ml. of 1% 4-CPA solution added to the suspension making it approximately 250 ml. of 100 ppm. 4-CPA. Suspension well shaken and sampled.

44a	103.4	92, 94,100,100,	37, 39, 44, 45,	5,7.5,	40
47	87.7	92, 92, 98, 99,	55, 56, 58, 59,	8, 10,	20
50	97.0	93, 94, 96, 99,	91, 93,101,104,	86, 89,	<1
53	97.1	96, 97,101,104,	95, 96, 99,106,	96,101,	<1

Day 56. 2.5 ml. of 1% 4-CPA solution added to the suspension making it approximately 250 ml. of 100 ppm. 4-CPA. Suspension well shaken and sampled.

56a	100.3	96,101,104,106,	33, 37, 37, 42,	5, 7,	50
59	102.3	88, 89, 92,109,	45, 47, 50, 53,	7, 8,	30
62	99.8	94,108,109,110,	101,105,107,117,	86, 87,	<1

Day 65. 2.5 ml. of 1% 4-CPA solution added to the suspension making it approximately 250 ml. of 100 ppm. 4-CPA. Suspension well shaken and sampled.

65a	95.8	94,102,108,110,	39, 41, 49, 56,	5,6.5,	45
68	96.3	97,100,105,127,	52, 52, 53, 60,	6,8.5,	25
71	94.0	90, 97, 98,106,	108,109,112,124,	89, 90,	<1

Day 74. 2.5 ml. of 1% 2,4-D solution added to the suspension making it approximately 250 ml. of 100 ppm. 2,4-D. Suspension well shaken and sampled.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	G.
74a	97.0	71, 74, 75, 82,	19, 20, 21, 27,	3, 5,	110
77	97.0	86, 88, 89, 95,	20, 21, 23, 33,	3, 3,	95
80	100.8	87, 88, 97, 98,	22, 23, 23, 25,	3, 4,	95

Table 21. Direct perfusion of 4-CPA, followed by 2-CPA.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Longest roots at nominal concentration of 10 ppm. as % m.c.
G. Indicated 4-CPA concentration in the perfusate (ppm.).
H. Indicated 2-CPA concentration in the perfusate (ppm.).

Perfusion started on 11/8/56 with 50 gm. of soil (2 to 4 mm. Sussex Lodge soil, dried February 1952,) and 250 ml. of 100 ppm. 4-CPA solution. First sample taken after 1 hr. perfusion.

A.	B.	C.	D.	E.	G.
0	67.5	67, 68, 70, 79,	24, 24, 28, 31,	4.5,4.5,	105
3	57.2	66, 73, 80, 80,	21, 21, 26, 26,	5, 5,	110
6	58.6	68, 68, 70, 73,	19, 21, 21, 24,	3.5, 5,	125
9	67.3	88, 89, 91,104,	34, 39, 45, 48,	9, 14,	50
12	61.9	87,104,105,115,	92,108,108,110,	104,128,	1
13b	57.1	103,103,119,137,	105,107,112,123,	84,130,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA. Perfusion restarted, sampled after 1 hr. Day 13.

13a	66.9	76, 85, 90, 93,	27, 27, 31, 43,	4.5, 6,	80
15	62.8	83, 84, 86,102,	94, 94, 97,105,	86, 88,	1
17b	43.4	88, 97,104,108,	99,101,108,111,	94,101,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA. Perfusion restarted, sampled after 1 hr. Day 17.

17a	43.4	76, 83, 94,106,	28, 28, 35, 35,	7, 7,	75
19	60.0	112,115,118,120,	93, 98,107,110,	98,107,	1
21b	76.6	60, 60, 64, 99,	75, 85, 88, 89,	64, 93,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA. Perfusion re-started, sampled after 1 hr. Day 21.

21a	71.9	54, 68, 72, 86,	18, 21, 25, 31,	4, 4,	110
23	72.0	100,107,107,111.,	107,109,113,121,	78,100,	1
25b	63.0	65, 68, 73, 91,	84, 84, 87, 99,	76, 92,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA. Perfusion restarted, sampled after 1 hr. Day 25.

25a	63.0	62, 62, 64, 87,	24, 25, 25, 27,	5,6.5,	100
27	66.9	90, 93,102,132,	63, 79, 81, 99,	76, 90,	1
29b	56.7	102,116,120,125,	74, 86, 92,116,	85, 88,	1

Table 21. continued.

Perfuser drained and refilled with 250 ml. of 10 ppm. 2-CPA.
Perfusion re-started, sampled after 1 hr. perfusion. Day 29.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	H.
29	56.7	53,	55,	65,	74,	44,	44,	49,	56,	23,	28,	8
34	40.1	60,	60,	60,	62,	47,	47,	52,	57,	40,	40,	8
39	52.8	97,	98,	102,	125,	53,	53,	57,	76,	25,	25,	5
44	54.6	79,	84,	84,	86,	60,	62,	62,	68,	29,	42,	4
49	54.6	82,	88,	93,	101,	44,	46,	55,	55,	20,	22,	8
54	63.5	86,	90,	95,	98,	36,	41,	44,	48,	20,	23,	9
59	67.4	104,	109,	110,	117,	43,	45,	49,	52,	24,	25,	6.5
64	66.4	84,	86,	89,	92,	51,	53,	54,	57,	16,	17,	9
69	78.3	93,	101,	104,	106,	56,	60,	64,	65,	24,	29,	4
74	76.4	87,	88,	96,	110,	49,	50,	51,	55,	20,	26,	6.5
79	65.4	110,	118,	122,	124,	37,	40,	49,	50,	29,	31,	6.5
84	65.2	104,	106,	106,	109,	68,	74,	86,	89,	28,	31,	3
89	65.0	82,	82,	86,	88,	32,	38,	42,	62,	17,	17,	10
94	66.7	77,	77,	78,	101,	32,	35,	38,	39,	21,	23,	5.5
99	75.1	79,	84,	85,	97,	65,	68,	68,	71,	25,	33,	3
104	76.2	87,	89,	98,	105,	64,	67,	72,	80,	20,	26,	3.5
109	63.7	83,	85,	86,	99,	55,	60,	60,	64,	24,	27,	5
114	45.3	75,	77,	88,	95,	53,	55,	55,	59,	29,	29,	6
119	81.6	79,	99,	103,	107,	37,	38,	42,	50,	24,	26,	7

Table 22. Direct perfusion of 4-CPA / 2-CPA mixture.

Key to columns in table:

A. Day of perfusion.
B. Mean control root length.(mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. (relative to the 4-CPA component) as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. (relative to the 4-CPA component) as % mean control.
E. Longest roots at nominal dilution concentration of 1.0 ppm. (relative to the 4-CPA component) as % mean control.
F. Indicated herbicide concentration in the perfusate as ppm. 4-CPA. (total activity).
G. Indicated 4-CPA concentration in the perfusate.(ppm.).

Perfusion.started on.8/2/53 with 50 gm. of.soil (2 to 4.mm. Sussex Lodge soil, dried January 1953,) and 250 ml. of solution containing 100 ppm. 4-CPA and 100 ppm. 2-CPA. Solution sampled before adding to the perfuser.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
0	84.2	68, 71, 79, 83,	18, 18, 20, 20,	3.5,4.5,	145
4	78.8	39, 42, 47, 56,	18, 22, 23, 24,	4, 5,	125
8	81.1	57, 60, 71, 75,	20, 21, 22, 23,	2.5,2.5,	125
11	82.4	44, 48, 48, 57,	21, 21, 22, 25,	3.5, 5,	125
14	83.5	68, 68, 78,102,	22, 24, 28, 35,	6, 7,	90
17	85.3	70, 78, 83, 88,	90, 92, 95, 97,	39, 42,	5
20	86.1	60, 63, 71, 84,	71, 75, 85, 87,	30, 34,	7
23	81.2	71, 72, 87, 91,	85, 88, 88,101,	53, 54,	2.5
26	75.9	90, 96,101,104,	84, 86, 95, 96,	30, 37,	6
29b	81.6	92,100,100,109,	84, 88, 89,105,	37, 49,	4

Perfuser drained and refilled with 250 ml. of solution containing 100 ppm 4-CPA and 500 ppm. 2-CPA. Perfuser re-started sampled after 1 hr. perfusion. Day 29.

29a	81.6	51, 67, 71, 82,	17, 19, 22, 32,	3.5, 5,	140
33	81.9	48, 49, 56, 56,	17, 21, 24, 30,	5,7.5,	120
37	72.8	86, 86, 89, 90,	62, 63, 66, 67,	27, 29,	15
41	72.5	69, 71, 77,101,	47, 51, 62, 66,	27, 29,	10
45	89.4	91, 93, 95, 99,	67, 69, 76, 80,	19, 33,	10
49b	88.3	81, 83, 87, 97,	59, 61, 68, 71,	24, 32,	10

Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 4-CPA and 1,000 ppm. 2-CPA. Perfuser re-started, sampled after 1 hr. perfusion. Day 49.

49a	85.2	41, 41, 42, 66,	17, 19, 21, 22,	3.5,4.5,	150
53	85.2	61, 64, 64, 70,	17, 19, 21, 21,	4.5, 8,	130
57	78.2	68, 73, 75, 80,	22, 22, 23, 26,	7.5,7.5,	110
61	82.3	65, 67, 72, 85,	42, 45, 46, 52,	19, 21,	22
65	80.0	66, 69, 71, 77,	38, 39, 46, 47,	19, 20,	20
69	74.9	101,101,104,108,	52, 53, 53, 61,	17, 19,	15

Table 22. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
77	82.0	70,	73,	73,	77,	44,	46,	51,	55,	17,	21,	20
82b	81.4	87,	87,	98,	101,	51,	51,	59,	60,	18,	22,	15

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr. Day 82.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
82a	74.5	71,	78,	78,	96,	20,	21,	21,	28,	5.5,	6.5,	110
84	74.5	88,	90,	91,	106,	22,	25,	30,	36,	4,	5.5,	90
86	83.9	100,	100,	105,	107,	33,	35,	35,	36,	8.5,	9.5,	50
88	84.3	86,	88,	92,	102,	94,	96,	98,	101,	45,	46,	4

Table 22a. Direct perfusion of 4-CPA / 2-CPA mixture.

Key to columns:
As in Table 22, above.
Details of perfusion set-up, as in Table 22, above.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	84.2	68,	71,	79,	83,	18,	18,	20,	20,	3.5,	4.5,	140
4	78.8	55,	56,	66,	80,	17,	18,	19,	20,	4,	4,	120
8	81.1	78,	91,	92,	101,	16,	16,	16,	22,	2.5,	3.5,	115
11	82.4	50,	53,	73,	79,	19,	22,	23,	32,	5,	6,	120
14	83.5	62,	72,	82,	82,	32,	36,	45,	45,	11,	11,	50
17	85.3	79,	81,	99,	103,	47,	48,	53,	71,	43,	51,	3
20	86.1	81,	84,	85,	89,	60,	72,	80,	82,	23,	33,	10
23	81.2	105,	106,	107,	117,	86,	86,	91,	91,	42,	42,	4
26	75.9	94,	96,	100,	120,	84,	86,	88,	89,	25,	28,	10
29b	81.6	98,	101,	105,	110,	81,	87,	93,	94,	43,	45,	4

Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 4-CPA and 500 ppm. 2-CPA. Perfuser re-started sampled after 1 hr. perfusion. Day 29.

29a	81.6	59,	62,	64,	78,	17,	17,	20,	23,	3.5,	5,	145
33	81.9	51,	55,	67,	68,	37,	37,	39,	48,	17,	22,	20
37	72.8	92,	96,	99,	100,	48,	51,	52,	52,	25,	27,	15
41	72.5	73,	81,	85,	92,	62,	67,	69,	77,	21,	23,	12
45	89.4	85,	85,	89,	98,	52,	57,	58,	58,	15,	17,	20
49b	88.3	87,	87,	105,	116,	42,	44,	46,	51,	21,	25,	10

Table 22a. continued.

Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 4-CPA and 1,000 ppm. 2-CPA. Perfuser re-started, sampled after 1 hr. perfusion. Day 49.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
49a	85.2	39,	39,	41,	55,	16,	17,	18,	20,	4.5,	8,	165
53	85.2	40,	41,	41,	43,	19,	19,	20,	25,	4.5,	6,	140
57	78.2	66,	66,	66,	74,	36,	37,	40,	49,	13,	14,	40
61	82.3	69,	70,	84,	87,	40,	44,	46,	52,	14,	17,	30
65	80.0	72,	76,	81,	87,	35,	36,	41,	44,	19,	23,	25
69	74.9	83,	93,	99,	103,	39,	41,	45,	45,	18,	23,	20
77	82.0	89,	91,	96,	103,	40,	42,	45,	46,	16,	19,	25
82b	81.4	87,	88,	101,	108,	47,	50,	55,	65,	22,	26,	17

Perfuser drained and refilled with 250 ml. of 100 ppm.4-CPA solution. Perfuser re-started, sampled after 1 hr. Day 82.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
82a	75.5	66,	71,	73,	82,	22,	24,	25,	36,	4,	6.5,	110
84	75.5	96,	96,	101,	110,	30,	33,	33,	37,	5.5,	9.5,	55
86	83.9	103,	110,	111,	113,	87,	97,	99,	107,	27,	29,	5
88	84.3	88,	89,	98,	100,	100,	101,	103,	104,	92,	94,	1

Table 23. Direct perfusion of 4-CPA, followed by 2,4-D.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Indicated 4-CPA concentration in the perfusate (ppm.).
G. Indicated 2,4-D concentration in the perfusate (ppm.).

Perfusion started on 11/5/52 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried April 1952,) and 250 ml. of 100 ppm. 4-CPA solution. First sample taken after 1 hr. perfusion.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
0	70.5	68, 80, 88, 97,	23, 24, 24, 40,	3,5.5,	110
3	48.3	92,108,108,145,	29, 33, 37, 37,	8.5,8.5,	55
6	68.2	60, 60, 63, 69,	21, 21, 21, 21,	6, 6,	125
9	66.9	78, 81, 87, 94,	28, 30, 36, 40,	7.5, 14,	60
12	67.0	97, 99,102,103,	81, 82, 88,108,	79, 82,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr. Day 15.

15a	61.6	82, 89, 98,111,	26, 26, 28, 31,	3.5, 8,	90
17	57.2	68, 84, 84,103,	51, 65, 70, 70,	14, 25,	15
19	57.6	83, 85, 97,146,	66, 71, 75, 97,	50, 64,	2

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr. Day 21.

21a	42.2	81, 83, 90, 97,	30, 30, 30, 43,	7, 7,	65
23	47.7	94,111,111,138,	75, 92,120,122,	55, 71,	2

Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr. Day 25.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	G.
25a	49.4	69, 75, 75, 97,	22, 26, 26, 28,	4, 6,	105
27	46.3	111,117,119,119,	104,108,110,117,	91, 97,	1
29	48.9	68, 70, 82, 84,	70, 84, 88, 88,	100,119,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr. Day 30.

30a	49.9	60, 64, 78, 94,	26, 34, 36, 40,	6, 6,	100
32	48.0	112,115,123,129,	65, 83,108,108,	96,135,	1
34	43.7	92, 94,112,123,	135,147,163,172,	101,112,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution, Perfuser re-started, sampled after 1 hr. Day 36.

Table 23. continued.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	G.
36a	51.4	78, 82, 95,123,	29, 33, 33, 35,	2, 4,	85
37	44.1	79, 86, 95,152,	34, 41, 48, 68,	11, 12,	30
38	44.1	89,111,123,152,	59, 73, 98,123,	50,134,	1

Table 23a. Direct perfusion of 4-CPA, followed by 2,4-D.

Key to columns:
As in Table 23, above.
Details of perfusion set-up, as in Table 23, above.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
0	70.5	80, 82, 85, 88,	26, 27, 30, 58,	1.5,4.5,	80
3	48.3	79,100,108,112,	29, 33, 35, 40,	8.5, 12,	65
6	68.2	75, 79, 79, 81,	16, 21, 21, 22,	4.5, 6,	90
9	66.9	75, 87, 94,108,	19, 21, 22, 24,	3, 6,	90
12	67.0	70, 84,102,126,	87, 90, 90,111,	57, 73,	2

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr. Day 15.

15a	61.6	91,102,111,132,	23, 23, 31, 33,	5,6.5,	90
17	57.2	89, 91, 94,101,	44, 47, 52, 62,	12, 19,	25
19	57.6	75, 97, 99,100,	54, 63, 68, 92,	49, 66,	2

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr. Day 21.

21a	42.2	90, 97, 97,102,	31, 36, 38, 40,	7,9.5,	50
23	47.7	74, 78, 80,118,	86,101,105,109,	75, 78,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr. Day 25.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	G.
25a	49.4	91,113,119,124,	26, 26, 26, 30,	6, 8,	100
27	46.3	61, 67, 69, 74,	108,108,115,180,	91, 99,	1
29	48.9	61, 68, 68,113,	100,106,115,164,	61, 70,	1

Perfuser drained and refilled with 250 ml. of 100. ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr. Day 30.

Table 23a. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
30a	49.9	64,	74,	80,	118,	26,	28,	32,	38,	4,	6,	100
32	48.0	83,	85,	96,	111,	60,	60,	65,	69,	12,	13,	25
34	43.7	128,	144,	149,	151,	117,	142,	147,	165,	144,	188,	<1

Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr. Day 36.

36a	51.4	70,	70,	105,	107,	29,	31,	33,	41,	6,	9.5,	90
37	44.1	86,	100,	111,	134,	36,	39,	43,	57,	7,	23,	50
38	44.1	116,	123,	129,	138,	100,	100,	120,	141,	79,	88,	<1

Table 23b. Direct perfusion of 4-CPA, followed by 2,4-D.

Key to columns in table:
As in Table 23, above.

Perfusion started on 5/2/52 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried June 1951,) and 250 ml. of 100 ppm. 4-CPA solution. First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	72.6	55,	56,	62,	74,	22,	25,	29,	33,	4,	4,	100
3	63.8	61,	63,	82,	103,	24,	24,	27,	31,	3,	4.5,	100
6	67.3	52,	64,	73,	85,	27,	30,	33,	34,	6,	14,	75
9	75.2	55,	67,	69,	93,	17,	18,	18,	19,	4,	5.5,	140
12	86.7	74,	76,	79,	82,	25,	28,	28,	32,	4.5,	9.5,	100
15	75.2	99,	105,	107,	123,	80,	80,	97,	109,	63,	69,	2
18	64.1	103,	109,	111,	116,	75,	76,	78,	80,	97,	106,	<1

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr. Day 20.

20a	63.7	64,	69,	72,	115,	24,	25,	25,	30,	4.5,	4.5,	100
22	58.1	69,	72,	79,	91,	44,	50,	55,	77,	8.5,	12,	50
24	55.1	112,	129,	135,	161,	87,	112,	124,	142,	71,	82,	<1

Perfuser drained and refilled with 250 ml of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr. Day 26.

26a	69.3	72,	81,	84,	93,	19,	20,	23,	28,	4.5,	5.5,	100
28	61.2	69,	70,	82,	95,	41,	41,	41,	56,	11,	15,	40

Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr. Day 30.

Table 23b. continued.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	G.
30a	71.1	70, 79, 82, 93,	15, 19, 23, 27,	3, 4,	120
32	69.4	76, 95,108,112,	88,101,103,112,	89,123,	<1
34	85.9	101,108,108,119,	108,108,112,119,	61, 71,	<1
36b	54.4	81, 96, 98, 99,	120,125,138,168,	96,110,	<1

Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr. Day 36.

36a	69.9	107,120,123,124,	23, 23, 26, 27,	3, 3,	115
38	77.5	94, 97,103,119,	56, 72, 74, 75,	45, 48,	15
40	54.4	103,105,110,114,	79, 83, 96,109,	98,109,	<1

Table 23c. Direct perfusion of 4-CPA, followed by 2,4-D.

Key to columns in table:
As in Table 23, above.
Details of perfusion set-up, as in Table 23b, above.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
0	72.6	78, 86, 90,102,	18, 19, 22, 23,	4,9.5,	135
3	63.8	80, 82, 85, 99,	15, 17, 17, 21,	3, 3,	125
6	67.3	67, 88, 94,106,	21, 21, 22, 28,	3, 3,	125
9	75.2	81, 84, 91,103,	17, 18, 19, 22,	4,5.5,	110
12	86.7	64, 64, 79,106,	17, 18, 21, 23,	2.5,3.5,	110
15	75.2	72, 76, 95,120,	33, 39, 41, 41,	5.5,5.5,	50
18	64.1	87, 93,103,104,	78, 84, 87,117,	70,116,	<1

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr. Day 20.

20a	63.7	55, 71, 75, 82,	20, 20, 20, 22,	4.5,4.5,	140
22	58.1	104,116,120,122,	36, 38, 38, 45,	5,8.5,	50
24	55.1	73, 76, 78, 91,	67, 76, 96,102,	27, 29,	10
26b	61.2	85, 95,120,129,	74, 79, 80,101,	79, 90,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr. Day 26.

26a	69.3	58, 71, 72, 98,	25, 25, 28, 38,	3,5.5	100
28	61.2	47, 52, 65, 98,	105,105,105,115,	87,118,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr. Day 30.

Table 23c. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
30a	71.1	84,	90,	97,	131,	17,	18,	18,	20,	3,	4,	120
32	69.4	96,	99,	109,	123,	93,	99,	101,	121,	46,	65,	4
34	85.9	94,	99,	107,	117,	97,	105,	110,	111,	41,	57,	4
36b	54.4	65,	68,	68,	74,	68,	94,	98,	134,	59,	81,	2

Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr. Day 36.

36a	69.9	99,	104,	106,	133,	29,	30,	31,	41,	1.5,	4.5,	90
38	77.5	77,	80,	94,	115,	93,	96,	103,	120,	41,	52,	5
40	54.4	131,	133,	140,	166,	110,	112,	135,	152,	79,	109,	1

Table 24. Direct perfusion of 4-CPA, followed by MCPA.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Indicated 4-CPA concentration in the perfusate (ppm.).
G. Indicated MCPA concentration in the perfusate (ppm.).

Perfusion started on 1/6/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 100 ppm. 4-CPA solution. First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	100.4	75,	75,	78,	89,	23,	24,	28,	29,	3,	3,	100
9	88.7	86,	90,	90,	95,	29,	30,	34,	46,	5.5,	6.5,	65
12	88.3	88,	88,	91,	92,	37,	38,	41,	45,	5,5,	9,	50
15	94.9	93,	94,	101,	102,	97,	99,	100,	108,	97,	109,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr. Day 18.

18a	103.4	87,	89,	90,	94,	36,	37,	39,	43,	5,	6,	80
21	101.7	90,	92,	93,	103,	92,	97,	98,	107,	90,	92,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr. Day 23.

23a	100.1	80,	86,	88,	116,	35,	37,	43,	48,	4,	7,	65
25	98.1	90,	90,	100,	103,	96,	99,	100,	106,	101,	104,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr. Day 27.

27a	102.9	91,	92,	92,	94,	33,	35,	36,	38,	4,	4,	55
29	102.7	87,	90,	94,	101,	96,	97,	99,	101,	95,	97,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr. Day 31.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
31a	97.5	68,	72,	86,	88,	18,	19,	21,	22,	2,	4,	90
34	96.6	81,	85,	93,	103,	19,	21,	25,	29,	2,	3,	65
37	105.6	73,	74,	77,	84,	20,	22,	27,	27,	3,	3,	70
40	98.6	78,	80,	83,	84,	23,	23,	23,	26,	2,	3,	65
46	95.9	85,	85,	86,	92,	24,	26,	32,	33,	4,	5,	50
52	99.7	82,	85,	90,	92,	31,	31,	33,	34,	3,	3,	45
58	101.2	89,	92,	93,	94,	25,	27,	29,	30,	4,	5,	50
61	98.4	86,	87,	90,	91,	34,	34,	37,	39,	4,	4,	45
67	93.8	95,	95,	97,	100,	43,	43,	46,	48,	6.5,	7.5,	25
70	105.1	97,	98,	100,	103,	52,	57,	62,	67,	8.5,	9.5,	20

Table 25. Direct perfusion of 4-CPA, followed by ∝-4-CPP.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Longest roots at nominal concentration of 10 ppm. as % m.c.
G. Indicated 4-CPA concentration in the perfusate (ppm.).
H. Indicated ∝-4-CPP concentration in the perfusate (ppm.).

Perfusion started on 28/7/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 100 ppm. 4-CPA solution. Solution sampled before adding to perfuser.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	G.
0	101.2	93, 96, 99,106,	40, 41, 46, 49,	6, 8,	40
6	99.4	90, 90, 99,101,	37, 38, 40, 42,	5, 7,	45
12	93.9	97,101,103,110,	100,100,103,106,	98,111,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr. Day 15.

15a	96.1	90, 90, 94, 97,	40, 41, 42, 43,	5, 6,	50
18	96.1	96, 97, 97, 99,	96, 99,100,112,	101,108,	1
20b	95.8	108,108,109,115,	102,103,109,111,	105,110,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr. Day 20.

20a	95.8	89, 92, 93, 97,	47, 47, 49, 49,	4, 5,	55
22	102.7	103,109,115,116,	103,104,105,109,	96, 99,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. ∝-4-CPP solution. Perfuser re-started, sampled after 1 hr. Day 24.

A.	B.	D. D. D. D.	E. E. E. E.	F. F.	H.
24a	95.2	44, 44, 47, 48,	13, 14, 17, 17,	2, 3,	110
27	95.2	91, 91, 97, 98,	99,103,104,111,	45, 50,	1
30b	99.1	91, 96, 96, 96,	96, 98, 98,100,	48, 50,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. ∝-4-CPP solution. Perfuser re-started, sampled after 1 hr. Day 30.

30a	97.0	45, 46, 49, 50,	17, 19, 19, 23,	3, 3,	100
33	97.0	90, 95, 97, 99,	81, 83, 86, 89,	29, 37,	2
36	97.3	98, 99,100,100,	87, 91, 93, 99,	42, 42,	1

Table 26. Direct perfusion of 4-CPA, followed by ∝-2,4-DCPP.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal dilution concentration of 0.1 ppm. as percentage mean control.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Longest roots at nominal concentration of 10 ppm. as % m.c.
G. Indicated 4-CPA concentration in the perfusate (ppm.).
H. Indicated ∝-2,4-DCPP concentration in the perfusate (ppm.)

Perfusion started on 28/7/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 100 ppm. 4-CPA solution. Solution sampled before adding to perfuser.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
0	101.2	93,	96,	99,	106,	40,	41,	46,	49,	6,	8,	40
6	99.4	83,	83,	85,	103,	41,	47,	47,	51,	7,	8,	35
12	93.9	97,	99,	99,	100,	99,	100,	101,	102,	102,	104,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr. Day 15.

15a	96.1	88,	89,	94,	95,	38,	42,	42,	50,	6,	8.5,	45
18	96.1	93,	93,	96,	99,	100,	101,	102,	108,	90,	102,	1
20b	95.8	99,	103,	111,	117,	105,	105,	105,	106,	99,	109,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr. Day 20.

20a	95.8	92,	94,	95,	110,	45,	47,	50,	59,	4,	4,	55
22	102.7	98,	100,	105,	116,	103,	104,	105,	108,	96,	102,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. ∝-2,4-DCPP solution. Perfuser re-started, sampled after 1 hr.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	H.
24a	100.2	28,	30,	31,	35,	4,	4,	5,	6,	1,	2,	100
29	100.2	24,	26,	29,	30,	4,	4,	5,	8,	2,	3,	100
34	99.1	26,	27,	30,	36,	6,	6,	7,	8,	1,	2,	85

Table 27. Direct perfusion of 4-CPA, followed by 4-CP.

Key to columns in table:

A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Indicated 4-CPA concentration in the perfusate (ppm.).
G. Colourimeter reading (Divs. on the UNICAM instrument).
H. Indicated 4-CP concentration in the perfusate.(ppm.).

Perfusion started on 8/2/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 100 ppm. 4-CPA solution. First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0-	84.2	78,	83,	89,	94,	21,	24,	26,	31,	6,	7,	100
4	78.8	58,	74,	79,	79,	18,	18,	20,	28,	4,	5,	105
8	81.1	60,	69,	70,	75,	18,	21,	22,	26,	2.5,	3.5,	125
11	82.4	69,	73,	75,	79,	40,	40,	50,	65,	8.5,	8.5,	50
14	83.5	90,	98,	103,	103,	69,	72,	76,	80,	12,	12,	10
17	85.3	91,	96,	105,	105,	104,	110,	112,	115,	93,	95,	1
20b	86.1	81,	89,	89,	94,	80,	81,	88,	90,	84,	86,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr. Day 20.

20a	86.1	69,	78,	84,	84,	22,	22,	26,	27,	4.5,	6,	110
23b	81.2	94,	96,	100,	117,	80,	90,	91,	95,	17,	23,	10

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr. Day 23.

23a	81.2	73,	73,	75,	75,	18,	19,	20,	25,	5,	6,	100
25	83.5	76,	78,	81,	86,	51,	61,	63,	68,	22,	30,	10
27b	67.8	75,	84,	87,	106,	83,	84,	85,	96,	99,	111,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr. Day 27.

27a	81.6	70,	80,	88,	91,	22,	24,	26,	26,	3.5,	5,	100
29	81.6	73,	84,	87,	90,	48,	49,	59,	67,	14,	16,	25
31b	92.9	90,	94,	99,	101,	86,	90,	90,	93,	46,	57,	5

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr. Day 31.

31a	92.9	57,	59,	70,	78,	20,	20,	22,	28,	3,	3,	125
33	81.9	65,	67,	67,	73,	46,	50,	60,	67,	11,	11,	25
35b	78.7	63,	81,	91,	96,	85,	91,	91,	98,	107,	117,	1

Table 27. continued.

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CP solution. Phenol solution sampled before adding to perfuser and again after 1 hr. perfusion. Day 35.

A.	G.	H.	
35ba	10.1	99	
35a	21.8	66	
36	28.4	54	
37	34.3	47	
38	46.9	33	
39	69.2	16	
40	92.0	4	
41b	89.8	5	Perfuser drained and refilled with 250 ml. of
41ba	10.6	97	100 ppm. 4-CP solution. Sampled before
41a	21.3	67	adding and again after 1 hr. perfusion.
42	29.2	53	
43	39.4	40	
44	48.8	31	
45	59.1	23	
46	70.9	15	
47	85.7	7	
48b	95.3	2	Perfuser drained and refilled with 250 ml. of
48ba	12.0	92	100 ppm. 4-CP solution. Sampled before
48a	20.6	69	adding and again after 1 hr. perfusion.
49	27.5	56	
50	31.0	51	
51	35.4	45	
52	38.5	42	
53	42.9	37	
54	51.2	29	
55	57.9	24	
56	60.2	22	
57	65.4	18	
58	71.9	14	
59	78.7	10	
60	85.8	7	
61	93.2	3	
62	94.4	3	
63	95.5	2	
64	98.8	1	
65	100.0	0	

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr. Day 67.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
67a	81.7	64, 76, 82, 92,	16, 17, 22, 26,	3.5, 5,	120
69	74.6	88, 96, 99,107,	44, 47, 48, 48,	10, 14,	30
71	73.9	83, 84, 93, 93,	83, 84, 84, 89,	60, 80,	1
73	80.5	112,112,116,123,	97,109,110,119,	97,104,	1

Table 27a. Direct perfusion of 4-CPA, followed by 4-CP.

Key to columns in table: as in Table 27, above.

Details of perfusion set-up: as in Table 27, above.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	84.2	78,	83,	89,	94,	21,	24,	26,	31,	6,	7,	100
4	78.8	61,	62,	68,	75,	15,	18,	19,	24,	4,	5,	130
8	81.1	79,	81,	94,	106,	15,	15,	16,	19,	3.5,	6,	125
11	82.4	77,	80,	88,	98,	40,	40,	41,	48,	8.5,	8.5,	70
14	83.5	75,	82,	85,	94,	80,	92,	92,	92,	38,	45,	5
17b	85.3	75,	79,	84,	102,	75,	78,	79,	101,	91,	102,	<1

Day 17. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr.

17a	85.3	60,	74,	80,	84,	16,	20,	22,	28,	4.5,	6,	120
20	86.1	70,	71,	81,	86,	88,	93,	96,	104,	67,	79,	1
23b	81.2	84,	95,	96,	100,	94,	96,	97,	110,	91,	93,	<1

Day 23. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr.

23a	81.2	70,	72,	80,	86,	20,	23,	26,	32,	5,	7.5,	105
25	83.5	73,	73,	74,	78,	49,	51,	51,	57,	9.5,	16,	30
27b	67.8	82,	93,	94,	96,	99,	100,	108,	127,	108,	115,	<1

Day 27. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr.

27a	81.6	67,	78,	81,	84,	22,	25,	25,	30,	3.5,	3.5,	100
29	81.6	87,	95,	100,	103,	83,	84,	88,	92,	97,	109,	<1
31b	92.9	84,	85,	87,	94,	83,	89,	91,	94,	75,	79,	<1

Day 31. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr.

31a	92.9	68,	76,	79,	86,	16,	19,	20,	22,	4.5,	5.5,	110
33	81.9	67,	71,	80,	90,	37,	40,	45,	55,	13,	20,	20
35b	78.7	70,	82,	89,	99,	76,	86,	90,	93,	94,	100,	<1

Day 35. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CP solution. Solution sampled before adding to the perfuser and after 1 hr. perfusion.

A.	C.	H.	A.	G.	H.	A.	G.	H.
35ba.	10.1	99,	37	33.2	48,	40	73.5	13,
35alhr.	21.5	67,	38	41.1	39,	41b.	95.1	2,
36	26.5	58,	39	53.8	27,			

Day 41. Drained and refilled with 250 ml. of 100 ppm. 4-CP.

41ba.	10.6	97,	43	37	43,	46	72.1	14
41alhr.	20.9	68,	44	47.3	32,	47	86.8	6,
42	29	54,	45	56.6	25,	48b.	95.5	2,

Table 27a. continued.

Day 48. Drained and refilled with 250 ml. of 100 ppm. 4-CP.

A.	G.	H.	A.	G.	H.	A.	G.	H.
48ba.	12	92,	51	39.2	41,	55	86.5	6,
48alhr.	20.5	69,	52	44.9	35,	56b.	93.4	3,
49	31.9	50,	53	53	28,			
50	34.1	47,	54	65.8	18,			

Day 56. Drained and refilled with 250 ml. of 100 ppm. 4-CP.

56ba.	15.7	80,	60	54	27,	64	94.4	2,
56alhr.	25.4	60,	61	64.1	19,	65	97.8	1,
57	33.8	47,	62	86.6	6,	66	100	0,
58	40	40,	63	92.6	3,	67b.	100	0,
59	45.9	34,	64					

Day 67. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
67a	81.7	45, 47, 48, 56,	19, 20, 22, 23,	5, 6,	130
69	74.6	99,104,108,115,	48, 50, 60, 71,	9.5, 13,	30
71	73.9	100,107,113,118,	89, 98,100,102,	23, 29,	10
73	80.5	78, 82, 88,100,	95, 98, 98,119,	109,112,	<1

Table 27b. Direct perfusion of 4-CPA, followed by 4-CP.

Key to columns in table: as in Table 27, above, except that the E.E.L. Colourimeter was used (Columns G. and H.).

Perfusion started on 11/5/52 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried April 1952,) and 250 ml. of 100 ppm. 4-CPA solution (Solution common with those of Tables 23, 23a, and 27c,). First sample taken after 1 hr. perfusion.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
0	70.5	75, 85, 85, 90,	18, 26, 26, 33,	4.5,4.5,	100
3	48.3	93,102,114,141,	35, 39, 48, 56,	8.5, 11,	40
6	68.2	69, 76, 78, 81,	21, 22, 26, 28,	3,4.5,	100
9	66.9	91,102,103,114,	24, 28, 28, 37,	4.5, 9,	90
12	67.0	97,102,105,106,	75, 79, 84, 96,	94,117,	<1

Day 15. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr.

15a	61.6	72, 80, 96,112,	26, 31, 37, 41,	5,6.5,	70

Table 27b. continued.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
17	57.2	63, 79, 93,145,	42, 44, 47, 49,	7, 7,	35
19	57.6	102,106,111,118,	92, 99,127,127,	70,111,	<1

Day 21. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr.

21a	42.2	78, 81, 88,100,	31, 33, 36, 43,	6, 8,	65
23	47.7	92,101,111,120,	84, 84, 92, 94,	40, 42,	5

Day 25. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CP solution. Sampled before adding and after 1 hr. perfusion.

A.	G.	H.	A.	G.	H.	A.	G.	H.
25ba.	77	103,	27	36.8	49,	30	15	20,
25alhr.	54.5	73,	28	27.6	37,	31b.	3.5	5,
26	45.5	60,	29	21	28,			

Day 31. Drained and refilled with 250 ml. of 100 ppm. 4-CP.

31ba.	71.5	95,	32	39.8	53,	34	10.4	14,
31alhr.	50.4	67,	33	25.8	34,	35b.	3.2	4,

Day 35. Drained and refilled with 250 ml. of 100 ppm. 4-CP.

35ba.	74	99,	36	34.3	46,	38b.	6.4	9,
35alhr.	49.5	66,	37	22.6	30,			

Day 38. Drained and refilled with 250 ml. of 100 ppm. 4-CP.

38ba.	71	95,	39	35	47,	41b.	3.2	4,
38alhr.	54	72,	40	19.1	25,			

Day 41. Drained and refilled with 250 ml. of 100 ppm. 4-CP.

41ba.	70	93,	42	/	/,	44b.	17	22,
41alhr.	49	65,	43	22.4	30,			

Day 44. Drained and refilled with 250 ml. of 100 ppm. 4-CP.

44ba.	77	102,	45	41	55,	47	21.4	28,
44alhr.	58.5	78,	46	31.8	42,	48b.	9.6	13,

Day 48. Drained and refilled with 250 ml. of 100 ppm. 4-CP.

48ba.	76.5	102,	49	26.2	35,
48alhr.	58	77,	50b.	9.0	12,

Day 50. Drained and refilled with 250 ml. of 100 ppm. 4-CP.

50ba.	79	105,	51	33.4	44,	53	3.1	4,
50alhr.	58	77,	52	6.5	12,			

Table 27c. Direct perfusion of 4-CPA, followed by 4-CP.

Key to columns in table: as in Table 27, above, except that the E.E.L. Colourimeter was used (columns G. and H.).

Details of perfusion set-up: as in Table 27b, above. Solution of 4-CPA common with those of Tables 23, 23a, and 27b,).

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
0	70.5	91, 98,104,111,	21, 23, 26, 28,	4.5,5.5,	100
3	48.3	52, 52, 67, 79,	23, 25, 27, 33,	6, 11,	100
6	68.2	68, 91, 96,107,	22, 25, 28, 28,	3, 6,	100
9	66.9	115,115,121,133,	25, 28, 30, 36,	4.5, 6,	80
12	67.0	82, 97,105,114,	54, 67, 72, 84,	79, 93,	<1

Day 15.Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr.

15a	61.6	91,106,114,116,	21, 26, 29, 34,	3.5, 5,	90
17	57.2	84, 87, 96,101,	28, 35, 35, 37,	5, 7,	55
19	57.6	83, 87, 94,101,	90, 92,125,148,	64, 96,	<1

Day 21. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr.

21a	42.2	76, 85, 92,100,	26, 29, 31, 31,	5, 6,	70
23	47.7	75, 92,109,138,	73, 82,103,107,	75, 88,	1

Day 25. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CP solution. Sampled before adding to the perfuser and after 1hr.

A.	G.	H.	A.	G.	H.	A.	G.	H.
25ba.	77	103,	28	21.2	29,			
25alhr.	54	72,	29	11	15,			
26	43	57,	30b.	3.5	5,			
27	32.7	43,						

Day 30. Drained and refilled with 250 ml. of 100 ppm. 4-CP.

30ba.	75	100,	31	36.6	49,	33b.	3	4,
30alhr.	49.5	66,	32	23.4	31,			

Day 33. Drained and refilled with 250 ml. of 100 ppm. 4-CP.

33ba.	74.5	99,	34	35	47,	36b.	7	9,
33alhr.	52	69,	35	20.2	27,			

Day 36. Drained and refilled with 250 ml. of 100 ppm. 4-CP.

36ba.	70	93,	37	38	51,	39b.	7.3	10,
36alhr.	53.2	71,	38	23	31,			

Table 27c. continued.

Day 39. Drained and refilled with 250 ml. of 100 ppm. 4-CP.

A.	G.	H.	A.	G.	H.	A.	G.	H.
39ba.	76	101,	40	46	61,	42	/	/,
39alhr.	58	77,	41	30	40,	43b.	10	13,

Day 43. Drained and refilled with 250 ml. of 100 ppm. 4-CP.

43ba.	74	98,	44	43.4	57,	46	20	27,
43alhr.	57	76,	45	31	41,	47b.	8.2	11,

Day 47. Drained and refilled with 250 ml. of 100 ppm. 4-CP.

47ba.	76.5	102,	48	41.5	55,	50b.	4.2	6,
47alhr.	57	76,	49	19	25,			

Day 50. Drained and refilled with 250 ml. of 100 ppm. 4-CP.

50ba.	79	105,	51	38	51,	53	1.9	3.
50alhr	53.5	71,	52	12.2	16,			

Table 28. Direct perfusion of 4-IPA, followed by 4-CPA.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Indicated 4-IPA concentration in the perfusate (ppm.).
G. Indicated 4-CPA concentration in the perfusate (ppm.).

Perfusion started on 1/4/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 100 ppm. 4-IPA solution. First sample taken after 1 hr. perfusion.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
0	82.5	90,100,104,108,	79, 84, 90, 98,	44, 45,	85
3	79.3	78, 79, 84, 86,	86, 91, 91,102,	35, 49,	95
6	86.7	90, 92,101,104,	84, 90, 94,104,	40, 48,	85
9	82.3	100,103,107,114,	85, 85, 92,102,	40, 47,	85
12	77.9	80, 82, 87, 97,	76, 78, 88,104,	39, 41,	100
15	77.7	76, 76, 88, 91,	83, 93,103,104,	49, 83,	85
18	74.9	69, 75, 79, 80,	76, 84,105,117,	36, 85,	75
21	80.5	90, 95,101,121,	97, 98,100,101,	41, 68,	50
24	70.7	114,123,124,130,	102,104,110,119,	82, 82,	15
27	78.5	107,107,113,121,	120,120,123,123,	80, 87,	15
30	75.5	95, 97,103,107,	92, 94, 98,108,	88, 90,	10
33	81.4	88, 91, 94, 96,	91, 91, 96, 98,	100,103,	1
36	80.6	106,108,110,118,	99,102,110,112,	98,100,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-IPA solution. Perfuser restarted, sampled after 1 hr. Day 39.

39a	88.6	94,100,102,102,	84, 87, 89, 89,	44, 46,	90
42	80.7	99,104,108,111,	94, 95, 99,104,	66, 72,	25
45	69.4	103,105,114,124,	96, 96, 99,102,	109,118,	1
48	87.6	95,100,106,114,	90,102,106,110,	96,109,	1
51b	88.3	97,104,105,107,	103,105,105,113,	101,104,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-IPA solution. Perfuser re-started, sampled after 1 hr. Day 51.

51a	88.3	92, 97, 98,102,	86, 92, 94,100,	42, 46,	80
54	89.8	96,102,104,106,	92, 98,102,104,	72, 76,	20
57	95.0	94, 99,101,101,	92, 96, 96,104,	100,106,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr. Day 60.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	G.
60a	96.9	86, 86, 88, 93,	22, 22, 26, 29,	5, 7,	90
62	93.9	85, 94,100,101,	47, 54, 56, 60,	13, 16,	25
64	92.3	100,102,104,113,	95, 95, 99,106,	100,107,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr. Day 66.

66a	95.7	88, 90, 91,102,	25, 27, 28, 28,	4, 5,	95
68	100.5	99,105,106,107,	83, 85, 88, 96,	38, 38,	5
70	95.2	86, 86, 91,103,	93, 99,108,111,	87, 87,	1

Table 29. Direct perfusion of 2,4-dichlorophenoxyacetic acid.

Key to columns in table:
- A. Day of perfusion.
- B. Mean control root length (mm.).
- C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
- D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
- E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
- F. Indicated 2,4-D concentration in the perfusate or suspension (ppm.).

Perfusion started on 22/12/50 with 50 gm. of soil (1 to 4 mm., Sussex Lodge soil, dried September 1950,) and 200 ml. of 1,000 ppm. 2,4-D solution. Solution sampled before adding to the perfuser and after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0ba.	33.5	60,	60,	66,	75,	18,	21,	27,	27,	3,	3,	1,100
0alhr	33.5	39,	60,	66,	72,	18,	21,	24,	27,	3,	3,	1,150
11	33.5	45,	48,	60,	72,	21,	27,	30,	42,	6,	6,	900
12	33.5	42,	96,	117,	132,	27,	27,	27,	27,	6,	6,	750
13	33.8	80,	92,	106,	118,	42,	54,	57,	62,	6,	6,	400
14	33.3	77,	81,	105,	118,	37,	40,	50,	65,	6,	6,	350
15	24.0	71,	75,	92,	108,	29,	29,	50,	67,	8.5,	13,	300
17	24.0	104,	108,	108,	117,	38,	42,	54,	71,	0,	8.5,	300
18	31.9	72,	75,	75,	88,	38,	41,	53,	75,	6,	6,	300
19	31.9	69,	100,	103,	116,	31,	38,	56,	75,	6,	9.5,	300
20	31.9	66,	85,	100,	106,	34,	56,	56,	63,	9.5,	9.5,	250
21	31.9	69,	91,	100,	109,	44,	44,	50,	56,	9.5,	9.5,	300
22	38.5	88,	91,	100,	107,	42,	49,	52,	65,	6.5,	11,	300
23	45.9	80,	87,	98,	109,	50,	52,	61,	70,	9,	9,	250
24	45.9	78,	98,	102,	113,	52,	65,	67,	87,	15,	24,	130
25	43.1	84,	98,	105,	114,	70,	95,	107,	116,	42,	46,	40
26	43.1	100,	102,	107,	118,	81,	86,	104,	114,	44,	63,	30
27	43.1	91,	104,	107,	109,	104,	107,	114,	118,	103,	104,	<10
28	31.5	79,	83,	124,	127,	83,	95,	111,	140,	60,	102,	<10
29	31.3	64,	83,	102,	128,	70,	90,	96,	118,	106,	112,	<10

Day 31. 20 ml. of 1% 2,4-D solution added to the remaining perfusate without draining. Volume made up to approximately 200 ml. with distilled water. Sampled after 1 hr. perfusion.

31a	26.6	41,	52,	90,	120,	52,	52,	64,	64,	7.5,	7.5,	300
32	32.8	64,	67,	82,	91,	46,	52,	61,	73,	9,	13,	250
33	36.3	85,	99,	102,	108,	41,	47,	50,	63,	8.5,	11,	300
34	36.3	74,	91,	96,	105,	39,	39,	50,	52,	7,	11,	300
35	33.8	83,	98,	112,	118,	44,	62,	71,	95,	7.5,	14,	250
36	35.8	81,	92,	104,	112,	78,	95,	98,	109,	39,	50,	40
37	35.3	111,	111,	114,	116,	65,	82,	102,	125,	94,	131,	<10
38	38.4	65,	81,	99,	104,	86,	86,	91,	144,	106,	106,	<10
39	39.4	66,	71,	74,	107,	79,	97,	104,	127,	104,	124,	<10
40	38.5	86,	96,	117,	135,	101,	104,	130,	166,	112,	133,	<10

Table 29. continued.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
41	38.5	101,117,125,127,	81,107,117,140,	112,120,	<10

Day 47. 20 ml. of 1% 2,4-D solution added to the remaining perfusate without draining. Volume made up to approximately 200 ml. with distilled water. Sampled after 1 hr. perfusion.

47a	34.7	81, 87, 90, 90,	40, 40, 46, 61,	9, 12,	450
48	34.7	87, 87, 92,107,	40, 52, 58, 63,	9, 23,	300
49	23.5	98,145,179,179,	34, 68, 77, 77,	13, 17,	150
50	26.8	89,100,104,112,	52, 60, 64, 75,	15, 22,	130
51	26.8	78, 86,101,119,	52, 56, 60, 60,	13, 19,	210
52	34.2	70, 91, 97, 99,	38, 44, 67, 73,	12, 21,	160
53	39.4	63, 66, 84, 89,	41, 51, 51, 71,	10, 23,	300
54	39.4	76, 86, 91, 96,	43, 48, 64, 76,	7.5, 10,	260
55	41.3	56, 65, 68, 85,	31, 31, 39, 41,	9.5,9.5,	450
56	29.2	86, 89, 92,123,	45, 48, 55, 65,	13, 14,	250
57	36.0	53, 67, 72, 75,	36, 39, 53, 72,	19, 25,	100
58	36.0	61, 64, 69,103,	56, 61, 64, 69,	17, 22,	120
59	36.0	72, 81, 92,100,	80, 89,100,103,	50, 55,	30
60	38.0	47, 55, 63, 68,	50, 55, 66, 74,	53, 76,	20
61	29.8	74, 74,101,104,	67, 88, 91,121,	124,151,	<10
62	29.8	57, 64, 77,107,	94,107,111,124,	155,161,	<10

Day 64. 20 ml. of 1% 2,4-D solution added to the remaining perfusate without draining. Volume made up to approximately 200 ml. with distilled water. Sampled after 1 hr. perfusion.

64a	39.8	68, 68, 78, 88,	33, 33, 38, 53,	5,7.5,	600
65	39.8	53, 68, 68, 70,	33, 33, 35, 40,	7.5,7.5,	600
66	35.0	57, 57, 80, 92,	34, 37, 40, 43,	5.5,5.5,	500
67	22.6	106,110,119,142,	49, 57, 57, 57,	9, 9,	250
68	31.1	64, 87, 96,106,	45, 48, 51, 51,	0,6.5,	300
69	31.1	71, 93, 96,106,	51, 51, 55, 64,	3,6.5,	300
70	31.1	77, 87, 90,128,	42, 45, 61, 67,	0,6.5,	300
71	30.0	83,100,113,127,	47, 53, 60, 77,	7, 10,	260
72	30.0	77, 80, 83,100,	50, 57, 60, 60,	10, 13,	210
73	30.0	70,100,120,133,	43, 47, 53,103,	13, 30,	110
74	30.1	97,113,120,137,	53, 83, 83, 97,	20, 37,	90
75	30.1	117,117,123,130,	73, 80, 87,107,	20, 43,	70
76	37.6	98,101,106,122,	66, 69, 72, 85,	13, 27,	120
77	37.6	96,101,106,130,	66, 72, 80,106,	19, 40,	80
78	37.6	88, 91,101,104,	59, 72, 85, 98,	24, 35,	70
79	23.5	77, 89, 94, 94,	72, 77, 77, 81,	34, 47,	50
80	23.5	85, 85,102,115,	85, 85, 85,102,	60, 64,	20
81	35.9	95, 95,100,106,	64, 78, 87, 87,	50, 58,	25
83	30.0	80, 83, 87, 90,	103,113,113,120,	70,100,	<10

Table 29. continued.

Day 85. 20 ml. of 1% 2,4-D solution added to the remaining perfusate without draining. Volume made up to approximately 200 ml. with distilled water. Sampled after 30 mins. perfusion.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
85a	29.3	68, 89, 96, 96,	48, 51, 65, 68,	7, 10,	350
86	36.3	69, 85, 91,118,	36, 39, 41, 64,	3,5.5,	700
87	36.3	55, 55, 69, 72,	33, 36, 47, 94,	3, 8,	600
88	36.3	72, 77, 88,110,	41, 44, 55, 64,	5.5,5.5,	500
89	36.3	55, 66, 74, 91,	30, 33, 50, 61,	8, 11,	450
102	36.3	74, 83, 96,149,	83, 88, 91, 99,	102,116,	10
103	36.3	61, 91,116,138,	83, 96,102,113,	110,132,	< 10

Day 109. 25 ml. of 1% 2,4-D solution added to the remaining perfusate without draining. Volume made up to approximately 250 ml. with distilled water. Sampled after 2 hr. perfusion.

109a	29.1	41, 52, 58, 79,	34, 34, 38, 65,	3.5, 7,	600
111	29.1	69, 76, 93, 93,	31, 34, 41, 58,	7, 10,	450
113	36.8	63, 76, 79, 84,	33, 38, 55, 71,	2.5,5.5,	500
115	36.8	79, 81, 90, 95,	33, 33, 41, 41,	5.5,5.5,	600
117	36.8	76, 81, 90,109,	30, 35, 38, 38,	5.5, 8,	500
119	31.3	83, 83, 90, 96,	32, 54, 64, 67,	6.5,6.5,	350
121	30.8	88, 88,117,120,	49, 52, 65, 72,	6.5, 10,	300
123	35.8	56, 64, 64, 92,	31, 34, 39, 39,	8.5,8.5,	500
125	17.6	108,125,131,136,	74, 74,102,102,	5.5,5.5,	550
127	36.7	55, 68, 68, 71,	52, 55, 63, 66,	5.5,5.5,	500

A new supply of Carter's Plain Cress seeds was used for assays beyond this point.

129	33.1	85, 85, 94,100,	39, 42, 42, 51,	6, 9,	450
131	29.3	92,116,120,137,	44, 51, 51, 58,	10, 14,	300
133	43.0	77, 86, 95,102,	42, 47, 53, 58,	7, 7,	400
135	46.2	56, 65, 72, 89,	24, 26, 28, 28,	6.5,6.5,	700
136	35.2	88, 94, 97,128,	31, 34, 43, 60,	8.5,8.5,	450
137	35.5	90, 99,104,104,	42, 45, 45, 48,	8.5, 11,	400
139	35.7	87, 90, 98,101,	31, 34, 39, 56,	5.5,8.5,	500
141	34.8	78, 81, 86,104,	38, 40, 55, 58,	6,8.5,	400
143	43.0	84, 89, 93,100,	28, 30, 46, 51,	4.5,4.5,	650
145	34.5	81, 81, 84, 96,	43, 43, 46, 55,	6,8.5,	500
149	45.3	73, 73, 73, 75,	35, 40, 49, 49,	6.5,6.5,	550
151	41.8	69, 88,100,103,	38, 38, 43, 50,	9.5, 15,	500
155	42.4	80, 83, 85, 92,	54, 59, 61, 78,	9.5, 12,	250
159	42.8	87, 87, 91,100,	66, 70, 72, 80,	40, 51,	40
163	45.2	80, 84, 91,100,	104,106,120,120,	95, 97,	< 10

Day 166. 25 ml. of 1% 2,4-D solution added to the remaining perfusate without draining. Volume made up to approximately 250 ml. with distilled water. Sampled after 1 hr. perfusion.

Table 29. continued.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
166	38.8	72, 75, 82, 95,	34, 39, 39, 57,	5, 5,	700
170	40.7	79, 86, 91, 91,	34, 44, 47, 54,	5,7.5,	450
174	32.8	70, 83, 83, 86,	52, 55, 58, 70,	9, 9,	300
178	32.8	98,110,113,132,	58, 58, 67, 83,	9, 9,	250
182	32.8	95,104,107,134,	49, 55, 67, 80,	6, 9,	250
186	32.8	110,116,119,131,	73, 80, 85, 91,	43, 46,	40
190	32.8	83, 85,104,104,	110,110,113,116,	100,122,	<10
192	43.7	108,110,117,140,	89, 92,110,112,	83, 96,	<10

Day 196. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr. perfusion.

196a	40.6	79, 79, 81, 86,	42, 49, 62, 74,	7.5, 10,	450
198	40.7	76, 76, 86,108,	42, 49, 54, 57,	5, 5,	450
200	37.2	83, 83, 89, 91,	59, 67, 67, 67,	8, 11,	350
202,	35.2	77, 80, 80, 85,	57, 60, 68, 77,	8.5,8.5,	300
204	38.5	68, 73, 91,114,	63, 68, 73, 83,	18, 21,	150
206	32.8	104,106,113,138,	92, 95, 98,106,	67, 76,	15
208	34.8	86, 86, 86,104,	86, 95,106,109,	83,100,	<10

Day 211. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr. perfusion.

211	36.6	90, 93, 96,101,	52, 55, 57, 66,	5.5, 8,	400
214	33.5	87, 87, 87,114,	54, 57, 63, 72,	12, 12,	250
216	38.4	65, 73, 76, 81,	47, 50, 52, 52,	8, 11,	350
218	33.6	104,110,116,131,	54, 57, 63, 63,	9, 15,	250
220	41.3	78, 90, 90,104,	51, 53, 56, 68,	20, 24,	180
222	42.8	84, 91, 91, 98,	66, 68, 70, 87,	14, 19,	160
224	43.6	83, 85, 90, 92,	62, 69, 71, 78,	18, 23,	150
226	40.1	125,127,130,132,	92, 95,100,110,	23, 35,	80
228	43.5	88, 94, 99,101,	60, 65, 67, 71,	35, 35,	60

Day 230. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr. perfusion. From this point, distilled water was used to make up the assay dilutions and in the control tubes.

230	64.2	84, 86, 90,108,	34, 44, 45, 51,	4.5, 6,	500
233	62.2	101,108,119,120,	29, 32, 34, 39,	5,6.5,	600
235	49.7	85, 93,109,127,	30, 32, 38, 46,	6, 6,	550
237	48.9	90, 92, 98,102,	33, 37, 37, 41,	6, 8,	500
239	67.3	77, 89,101,116,	24, 27, 28, 45,	6, 6,	550
241b	64.1	95,100,101,104,	30, 33, 42, 67,	6, 8,	400

Day 241. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr. perfusion.

Table 29. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
241a	64.1	67,	69,	70,	112,	25,	27,	27,	56,	4.5,	6,	800
246	77.7	72,	76,	89,	91,	21,	22,	23,	26,	4,	4,	1,000
248	67.1	78,	107,	107,	136,	27,	28,	33,	39,	3,	6,	800
250	74.8	70,	72,	82,	84,	20,	21,	21,	31,	4,	4,	1,050
252	70.1	74,	80,	93,	103,	24,	24,	27,	31,	4,	6,	900
254	56.4	71,	76,	78,	78,	21,	23,	25,	28,	3.5,	3.5,	950
256	60.1	87,	90,	95,	102,	23,	23,	28,	28,	5,	5,	750
258	51.6	87,	89,	105,	110,	31,	33,	35,	37,	6,	8,	600
260	42.3	99,	107,	120,	124,	33,	38,	40,	40,	7,	14,	450
262	45.4	82,	84,	106,	106,	31,	33,	38,	38,	4.5,	6.5,	500
264	55.0	80,	82,	89,	100,	29,	29,	33,	33,	5.5,	7.5,	650
266	56.0	79,	81,	84,	91,	29,	29,	29,	32,	3.5,	5.5,	700
268	62.6	85,	88,	91,	123,	24,	27,	27,	32,	5,	6.5,	800
270	66.5	83,	83,	88,	89,	26,	27,	27,	27,	4.5,	4.5,	800
272	76.3	62,	69,	87,	90,	24,	24,	28,	30,	6.5,	9,	800
274	68.8	70,	80,	80,	89,	29,	35,	39,	47,	4.5,	4.5,	650
276	57.5	71,	87,	92,	102,	33,	37,	40,	42,	5,	7,	500
278	69.3	64,	64,	68,	72,	50,	50,	56,	71,	8.5,	13,	300
280	46.0	74,	76,	81,	81,	67,	72,	74,	85,	26,	28,	80
282	72.3	75,	86,	89,	97,	82,	89,	96,	98,	83,	114,	<10
284	54.9	100,	122,	122,	133,	64,	64,	66,	80,	47,	62,	30

Day 285. Perfuser drained and refilled with 250 ml; of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr. perfusion.

285a	67.6	56,	68,	83,	93,	21,	25,	25,	31,	3,	3,	900
291	72.3	84,	84,	90,	102,	22,	24,	24,	28,	4,	5.5,	950
293	59.8	87,	87,	92,	109,	25,	30,	33,	35,	3.5,	5,	800
295	59.3	67,	69,	85,	98,	29,	32,	47,	52,	6.5,	10,	650
297	67.7	71,	74,	74,	83,	24,	33,	34,	52,	7.5,	11,	600
299	69.9	91,	96,	106,	107,	46,	51,	60,	61,	9,	10,	250
301	36.5	66,	71,	107,	132,	49,	63,	74,	80,	8,	11,	200
303	60.9	59,	84,	91,	102,	41,	48,	66,	76,	20,	21,	150
305	51.7	70,	83,	102,	118,	93,	99,	100,	116,	56,	70,	20
307	53.3	60,	77,	83,	85,	70,	77,	94,	96,	66,	88,	10

Day 309. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr. perfusion.

309a	52.3	71,	71,	98,	122,	25,	25,	31,	38,	4,	4,	900
312	64.0	72,	80,	86,	100,	20,	27,	30,	33,	4,	5.5,	750
314	60.7	87,	94,	102,	104,	33,	35,	35,	44,	5,	6.5,	600
316	66.8	85,	85,	102,	108,	21,	27,	27,	30,	4.5,	6,	800
318	63.8	83,	83,	86,	97,	31,	31,	31,	42,	4.5,	4.5,	700
320	71.5	80,	81,	87,	90,	21,	22,	28,	48,	3,	4,	1,000
322	70.7	81,	86,	91,	100,	27,	27,	31,	37,	5.5,	7,	750

Table 29. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
324	62.8	72,	91,	92,	110,	30,	35,	38,	46,	5,	6.5,	600
326	75.2	83,	85,	88,	97,	27,	28,	32,	36,	2.5,	4,	800
328	79.1	69,	71,	76,	77,	28,	28,	34,	43,	2.5,	4,	800
330	75.2	84,	84,	97,	127,	27,	29,	31,	43,	4,	8,	600
332	81.6	60,	75,	80,	95,	23,	23,	28,	32,	3.5,	5,	950
334	69.5	66,	66,	74,	84,	17,	19,	20,	26,	4.5,	4.5,	1,000
336	59.4	74,	74,	74,	123,	25,	25,	29,	29,	5,	6.5,	700
338	55.9	100,	104,	111,	117,	29,	32,	32,	36,	5.5,	5.5,	650
340	68.3	78,	88,	91,	102,	18,	19,	19,	35,	6,	9,	750
342	66.0	87,	93,	101,	102,	24,	29,	29,	38,	3,	4.5,	750
344	67.4	73,	76,	94,	98,	27,	27,	28,	34,	6,	7.5,	800
347	67.6	71,	87,	92,	95,	21,	24,	25,	28,	4.5,	8.5,	900
350	76.3	72,	75,	80,	113,	20,	20,	24,	30,	2.5,	4,	950
352	70.3	53,	54,	69,	80,	21,	21,	26,	29,	7,	8.5,	800
355	72.4	90,	91,	102,	107,	23,	32,	36,	48,	3,	4,	600
358	66.9	79,	84,	96,	118,	24,	27,	33,	45,	7.5,	15,	600
373	73.3	72,	75,	83,	86,	36,	37,	42,	57,	5.5,	5.5,	550
376	81.3	84,	90,	101,	108,	53,	64,	72,	74,	12,	15,	200
379	77.7	89,	100,	106,	116,	70,	71,	81,	88,	44,	80,	25
382	72.0	92,	97,	102,	118,	61,	63,	74,	82,	107,	118,	<10
384b	71.6	71,	87,	90,	126,	98,	101,	105,	109,	68,	83,	<10

Day 384. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr. perfusion.

384a	60.8	67,	67,	69,	79,	28,	28,	28,	35,	8,	10,	800
389	66.3	83,	96,	106,	109,	26,	26,	26,	29,	4.5,	9,	800
392	66.3	75,	78,	80,	80,	20,	20,	23,	26,	3,	4.5,	1,000
395	51.2	68,	80,	86,	88,	33,	35,	35,	41,	6,	10,	600
398	49.9	98,	102,	112,	120,	38,	46,	50,	66,	6,	10,	350
401	77.7	71,	92,	99,	108,	45,	48,	49,	60,	9,	12,	300
404	86.6	65,	71,	81,	85,	58,	58,	74,	78,	22,	22,	100
407	46.6	101,	105,	112,	144,	84,	86,	88,	90,	120,	134,	<10

Day 411. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

411a	61.4	47,	53,	60,	63,	14,	14,	15,	18,	3.5,	5,	180
414	68.4	83,	88,	91,	104,	63,	63,	66,	79,	28,	29,	10
416	67.3	79,	86,	106,	107,	66,	67,	69,	70,	66,	73,	1.5
418b	87.3	65,	71,	77,	103,	91,	95,	98,	98,	87,	88,	<1

Day 418. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

418a	87.3	57,	60,	69,	78,	15,	16,	18,	20,	2.5,	3.5,	150
420	66.4	61,	64,	79,	102,	21,	21,	22,	26,	2,	3,	110
422b	86.7	47,	49,	84,	91,	81,	85,	87,	87,	81,	82,	<1

Table 29. continued.

Day 422. Perfuser had ceased to function mechanically. Entire contents of column (glass-wool plugs and soil) were placed in a sterile plugged flask with 250 ml. of sterile 100 ppm. 2,4-D solution. The suspension was incubated at 28°C with occasional shaking.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
422a	83.8	63, 69, 77, 95,	19, 20, 22, 25,	3.5,8.5,	115
424	75.2	71, 72, 72, 85,	31, 32, 33, 35,	4,5.5,	65
426	69.5	88,102,102,102,	29, 30, 30, 33,	4.5,8.5,	65
428	64.1	108,110,110,111,	30, 39, 45, 48,	6.5,9.5,	40,
430	63.7	66, 68, 68, 93,	27, 28, 39, 41,	4.5, 11,	50
432	58.1	104,129,148,148,	31, 34, 36, 57,	7,9.5,	45
434	55.1	85,91,93, 105,	44, 45, 53, 55,	12, 15,	25
436	69.3	94, 94,100,123,	38, 49, 49, 58,	6, 7,	30
440	71.1	80, 80, 96,118,	46, 46, 48, 55,	7,8.5,	35
449	54.4	90,111,111,138,	72, 74, 77,112,	16, 19,	15
454	63.1	94,106,117,121,	78,108,113,113,	62, 89,	1.5
458	69.6	69, 72, 97,102,	72, 73, 94, 96,	59, 96,	<1

Day 461. 2.5 ml. of 1% 2,4-D solution and a little water added to the suspension making the volume approximately 250 ml. of 100 ppm. 2,4-D. Suspension shaken, sampled after 1 hr.

461a	56.0	86, 98,100,142,	18, 19, 30, 32,	7, 9,	95
464	68.3	53, 56, 59, 63,	25, 25, 29, 37,	4.5,7.5,	75
467	68.5	92, 95,101,117,	23, 28, 31, 34,	6, 9,	60
470	59.8	64, 67, 69, 79,	32, 37, 40, 45,	4,8.5,	50
473	79.0	70, 82, 85, 93,	39, 44, 48, 51,	4,6.5,	40
492	69.8	94,109,110,123,	83, 96,104,112,	53, 56,	3
494b	67.0	100,114,118,121,	97,100,108,111,	70, 90,	<1

Day 494. 2.5 ml. of 1% 2,4-D solution added to the suspension making it approximately 250 ml. of 100 ppm. 2,4-D. Suspension was shaken, sampled after 1 hr. perfusion.

494a	66.8	81, 83, 87, 99,	13, 14, 17, 20,	3, 6,	120
497	73.5	95, 95, 95,124,	26, 26, 39, 44,	4,5.5,	60
502	64.8	80, 85, 88,100,	40, 45, 56, 60,	6,7.5,	40
507	56.6	114,118,120,122,	37, 41, 65, 81,	12, 18,	20
512	68.2	75, 78,101,101,	51, 69, 75, 76,	38, 44,	5
517	66.6	65, 75, 86, 95,	57, 59, 66, 92,	62, 80,	1.5

Day 522. 2.5 ml. of 1% 2,4-D solution and a little water added to the suspension making it approximately 250 ml. of 100 ppm. 2,4-D. Suspension shaken, sampled after 1 hr.

522a	61.6	78, 81, 88, 99,	21, 23, 24, 24,	3.5,3.5,	95

Table 29. continued.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
527	42.2	85, 85,107,107,	43, 43, 45, 52,	9.5, 14,	40
532	44.0	91, 93, 95,100,	36, 41, 43, 61,	7, 9,	40
537	41.3	87, 92, 92,126,	44, 56, 75, 85,	14, 17,	20
542	51.4	49, 53, 62, 88,	47, 62, 80, 90,	13, 16,	20
547	52.5	48, 53, 76, 82,	65, 89,109,126,	17, 21,	10
552	59.4	98,103,114,133,	98,101,114,119,	44, 59,	3
557b.	40.7	103,106,113,140,	118,121,138,153,	116,143,	<1

Day 557. 2.5 ml. of 1% 2,4-D solution and a little water added to the suspension making it approximately 250 ml. of 100 ppm. 2,4-D. Suspension shaken and sampled after 1 hr.

557a.	40.7	103,118,138,155,	32, 35, 37, 44,	10, 15,	55
562	52.5	53, 53, 72, 91,	30, 36, 38, 40,	4,9.5,	50
567	50.5	87, 95,107,113,	38, 42, 51, 61,	18, 32,	30
572	52.1	88,108,111,150,	48, 54, 73, 75,	17, 17,	20
577	46.8	90, 98,109,113,	49, 62, 66, 90,	15, 24,	17
582	59.4	91,108,110,110,	59, 71, 72, 93,	25, 27,	10
597	50.9	88, 92,120,120,	90,102,114,132,	104,126,	<1

Day 602. 2.5 ml. of 1% 2,4-D solution and a little water added to the suspension making it approximately 250 ml. of 100 ppm. 2,4-D. Suspension shaken and sampled after 1 hr.

602a	55.7	67, 72, 77, 81,	23, 27, 29, 32,	5.5,5.5,	90
607	67.3	62, 67, 70, 94,	24, 24, 27, 34,	4.5, 6,	85
612	66.9	90, 90,108,114,	37, 39, 45, 45,	9, 15,	40
617	60.0	85, 90, 95, 98,	82, 82, 88, 93,	27, 32,	10
622b	54.9	88, 97,106,109,	97,106,109,122,	36, 40,	5

Day 622. 2.5 ml. of 1% 2,4-D solution and a little water added to the suspension making it approximately 250 ml. of 100 ppm. 2,4-D. Suspension shaken and sampled after 1 hr.

622a	54.9	66, 73, 77, 86,	24, 24, 31, 36,	3.5,3.5,	90
627	71.8	61, 75, 75, 89,	17, 18, 18, 21,	5.5, 7,	70
632	40.1	92, 95, 95,112,	37, 40, 47, 57,	7.5, 10,	40
637	52.8	53, 57, 64, 91,	28, 28, 30, 36,	9.5, 12,	30
647	51.2	98,104,104,111,	86, 96,100,107,	123,129,	<1
651b	61.6	88, 91,107,112,	89, 96,104,104,	83,101,	<1

Day 651. 2.5 ml. of 1% 2,4-D solution added to the suspension making it approximately 250 ml. of 100 ppm. 2,4-D. Suspension shaken and sampled after 1 hr.

651a	61.6	50, 52, 57, 84,	24, 24, 26, 31,	5, 5,	90
661	64.8	69, 74, 80,102,	89, 91, 96,103,	66, 88,	1
666b	80.3	111,112,119,120,	100,100,101,103,	96, 97,	<1

Table 29. continued.

Day 666. 2.5 ml. of 1% 2,4-D solution added to the suspension making it approximately 250 ml. of 100 ppm. 2,4-D. Suspension shaken and sampled after 1 hr.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
666a	80.3	69, 71, 72, 96,	26, 27, 27, 30,	5,7.5,	80
671	75.0	92, 93, 97, 97,	37, 43, 43, 61,	13, 15,	40
676	65.4	96,109,112,118,	70, 72, 80, 83,	28, 29,	10
681b	67.8	90, 90, 90,112,	88, 97,100,106,	84, 99,	<1

Day 681. 2.5 ml. of 1% 2,4-D solution and a little water added to the suspension making it approximately 250 ml. of 100 ppm. 2,4-D. Suspension shaken and sampled after 1 hr.

681a	67.8	56, 58, 60, 88,	16, 17, 18, 24,	[illegible]3, [illegible]3,	135
686	57.7	90, 94, 97,107,	38, 43, 49, 61,	8.5, 16,	35
691	64.7	97, 97, 99,100,	108,116,117,119,	105,111,	1
696b	73.1	88, 92, 99,105,	66, 68, 82, 97,	49, 52,	<3

Day 696. 2.5 ml. of 1% 2,4-D solution added to the suspension making it approximately 250 ml. of 100 ppm. 2,4-D. Suspension shaken and sampled after 1 hr.

696a	73.1	64, 67, 79, 92,	30, 30, 32, 38,	7, 8,	70
701	76.2	72, 72, 77, 83,	33, 34, 37, 41,	6.5, 11,	55
706	77.9	82, 82, 86, 89,	40, 42, 45, 58,	8.5, 12,	40
711	58.3	89, 93, 95,108,	60, 69, 77, 79,	15, 17,	15
716	68.0	78, 81,100,111,	94, 94, 96,102,	57, 65,	2
721b	85.1	76, 78, 82, 91,	89,103,106,107,	92, 96,	<1

Day 721. 2.5 ml. of 1% 2,4-D solution and a little water added to the suspension making it approximately 250 ml. of 100 ppm. 2,4-D. Suspension shaken and sampled after 1 hr.

721a	75.4	82, 89, 91,102,	20, 25, 25, 32,	2.5, 4,	85
726	77.1	84, 88, 88, 89,	26, 32, 34, 39,	4, 6,	65
751	65.3	86, 87, 90, 92,	72, 87,107,110,	75, 89,	1

Day 756. 2.5 ml. of 1% 2,4-D solution and a little water added to the suspension making it approximately 250 ml. of 100 ppm. 2,4-D. Suspension sampled 1 hr. after addition and shaking.

756a	82.8	52, 59, 68, 69,	17, 17, 18, 21,	2.5, 5,	135
761	76.1	87, 87, 87, 94,	30, 34, 38, 41,	12, 17,	55
766b	75.3	91, 95,102,107,	91, 95,103,109,	48, 49,	3

Day 766. 2.5 ml. of 1% 2,4-D solution added to the suspension making it approximately 250 ml. of 100 ppm. 2,4-D. Suspension sampled 1 hr. after addition and shaking.

766a	75.3	62, 65, 65, 67,	18, 19, 20, 22,	2.5, 4,	125

Table 29. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
776	85.9	87,	88,	93,	97,	90,	93,	95,	97,	21,	23,	10
781b	83.8	69,	85,	91,	104,	71,	71,	83,	83,	69,	70,	1.5

Day 781. 2.5 ml. of 1% 2,4-D solution and a little water added to the suspension making it approximately 250 ml. of 100 ppm. 2,4-D. Suspension shaken and sampled after 1 hr.

781a	83.8	67,	68,	69,	69,	21,	24,	24,	27,	3.5,	3.5,	95
786	77.4	76,	76,	78,	81,	32,	35,	44,	50,	13,	14,	50
791	79.3	72,	74,	77,	87,	79,	80,	83,	93,	29,	43,	6
796	85.3	84,	84,	88,	93,	88,	91,	93,	101,	88,	97,	<1

Day 796. 2.5 ml. of 1% 2,4-D solution and a little water added to the suspension making it approximately 250 ml. of 100 ppm. 2,4-D. Suspension shaken and sampled after 1 hr.

796a	85.3	55,	63,	63,	64,	16,	17,	20,	21,	2.5,	3.5,	150
801	85.3	64,	67,	73,	81,	30,	32,	35,	39,	7,	8,	60
806	67.8	84,	96,	112,	119,	75,	85,	90,	91,	79,	81,	1
811b	88.4	79,	85,	88,	92,	79,	80,	80,	80,	57,	65,	2.5

Day 811. 2.5 ml. of 1% 2,4-D solution added to the suspension making it approximately 250 ml. of 100 ppm. 2,4-D. Suspension shaken and sampled after 1 hr.

811a	88.4	53,	59,	59,	62,	14,	15,	17,	20,	3.5,	3.5,	165
816	72.8	71,	71,	84,	92,	51,	52,	64,	68,	11,	15,	20
821	73.9	89,	100,	108,	111,	93,	95,	96,	97,	91,	91,	<1
826b	77.8	107,	109,	109,	118,	83,	83,	96,	121,	80,	86,	<1

Day 826. 2.5 ml. of 1% 2,4-D and a little water added to the suspension making it approximately 250 ml. of 100 ppm. 2,4-D. Suspension shaken and sampled after 1 hr.

826a	77.8	65,	76,	79,	87,	19,	21,	22,	23,	4,	4,	105
831	82.5	82,	94,	100,	103,	54,	64,	68,	71,	13,	16,	20
836	78.2	92,	93,	98,	101,	98,	100,	106,	112,	109,	115,	<1

Day 841. 2.5 ml. of 1% 2,4-D solution added to the suspension making it approximately 250 ml. of 100 ppm. 2,4-D. Suspension shaken and sampled after 1 hr.

841a	76.0	55,	56,	79,	80,	17,	18,	20,	24,	4,	5.5,	115
846	77.7	83,	85,	90,	95,	37,	38,	40,	42,	13,	16,	50
851	82.1	83,	83,	89,	91,	107,	108,	111,	113,	104,	108,	1

Day 856. 2.5 ml. of 1% 2,4-D solution added to the suspension making it approximately 250 ml. of 100 ppm. 2,4-D. Suspension shaken and sampled after 1 hr.

Table 29. continued.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
856a	69.9	89, 94,110,110,	24, 24, 26, 33,	5.5, 7,	90
861	75.5	50, 51, 54, 55,	30, 33, 38, 38,	5.5,6.5,	55
866	80.6	105,107,108,110,	99, 102,104,108,	89, 98,	<1

Day 871. 2.5 ml. of 1% 2,4-D solution added to the suspension making it approximately 250 ml. of 100 ppm. 2,4-D. Suspension shaken and sampled after 1 hr.

871a	80.7	89, 89, 93, 93,	22, 24, 25, 26,	3.5, 5,	90
876	69.4	106,115,116,119,	36, 37, 52, 53,	10, 13,	40
881	85.3	91, 91, 92,104,	98, 99,104,104,	83, 95,	<1

Day 886. 2.5 ml. of 1% 2,4-D solution and a little water added to the suspension making it approximately 250 ml. of 100 ppm. 2,4-D. Suspension shaken and sampled after 1 hr.

886a	92.6	72, 72, 77, 77,	21, 21, 25, 26,	4.5,5.5,	105
891	86.9	87, 93, 94, 97,	95,102,103,107,	88,101,	<1
896b	98.3	94, 95,100,101,	94, 94, 95,107,	103,106,	<1

Day 896. 2.5 ml. of 1% 2,4-D solution and a little water added to the suspension making it approximately 250 ml. of 100 ppm. 2,4-D, Suspension shaken and sampled after 1 hr.

896a	98.6	84, 84, 84, 87,	21, 22, 23, 26,	3, 3,	105
901	95.2	86, 87, 98,100,	65, 65, 81, 84,	19, 20,	10
906	91.7	100,103,107,112,	97, 98,100,100,	88, 95,	<1

Day 911. 2.5 ml. of 1% 2,4-D solution added to the suspension making it approximately 250 ml. of 100 ppm. 2,4-D. Suspension shaken and sampled after 1 hr.

911a	103.4	90, 91, 93, 95,	22, 22, 22, 23,	3, 4,	100
916	100.1	96, 97,102,106,	59, 63, 63, 65,	11, 13,	20
921b	102.7	88, 90, 90, 98,	84, 84, 86, 90,	20, 33,	9

Day 921. 2.5 ml. of 1% 2,4-D solution added to the suspension making it approximately 250 ml. of 100 ppm. 2,4-D. Suspension shaken and sampled after 1 hr.

921a	102.7	80, 81, 84, 95,	19, 20, 20, 22,	4, 4,	110,
926	96.6	81, 84, 95,101,	29, 30, 37, 39,	5, 6,	65
931	99.9	93, 93, 94, 97,	50, 52, 52, 58,	7, 8,	45
936	99.6	87, 88, 91, 92,	42, 43, 45, 47,	6, 7,	55
941	98.4	88, 91, 93, 97,	58, 59, 62, 66,	6.5,9.5,	35
946	100.1	106,107,109,119,	72, 73, 75, 75,	8, 8,	15
951	87.7	98,100,104,106,	101,106,108,111,	60, 61,	2

Day 956. 2.5 ml. of 1% 2,4-D solution added to the suspension making it approximately 250 ml. of 100 ppm. 2,4-D. Suspension shaken and sampled after 1 hr.

956a	97.1	82, 85, 88, 93,	27, 33, 36, 39,	5, 7,	60
961	102.3	101,102,102,107,	43, 43, 54, 57,	6, 6,	40
966	99.8	88, 95, 95,115,	49, 54, 54, 59,	8, 9,	30
971	102.7	95, 97,100,101,	94, 98, 99,102,	102,107,	<1

Table 29a. Breakdown of 2,4-D in soil-less suspension.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Indicated 2,4-D concentration in the suspension (ppm.).

Incubation started on 16/1/53 with 250 ml. of solution, containing 200 ppm. 2,4-D, prepared from 25 ml. of clear liquid from above an active soil suspension (Table 29,), distilled water, and 0.05 gm. KCl, 0.05 gm. $MgSO_4$, 0.25 gm. $(NH_4)H_2PO_4$. Suspension shaken, sampled, then incubated at 28°C.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	82.8	59,	60,	60,	72,	14,	14,	16,	21,	2.5,	2.5,	200
5	76.1	42,	42,	43,	55,	16,	17,	17,	21,	2.5,	4,	145
10	75.3	49,	52,	53,	64,	17,	18,	19,	27,	5.5,	5.5,	130
20	85.9	60,	66,	67,	73,	14,	15,	15,	15,	2.5,	3.5,	170
25	83.8	43,	49,	52,	54,	18,	18,	19,	20,	2.5,	3.5,	130
30	77.4	49,	63,	68,	71,	13,	13,	14,	14,	2.5,	4,	200
35	79.3	44,	48,	57,	63,	14,	14,	15,	17,	4,	5,	190
40	85.3	56,	62,	65,	75,	14,	15,	15,	23,	2.5,	3.5,	170
45	85.3	34,	39,	41,	43,	12,	13,	15,	18,	4.5,	6,	130
50	91.3	57,	61,	69,	81,	15,	16,	18,	20,	3.5,	5.5,	150
55	88.4	59,	59,	61,	62,	25,	25,	26,	43,	3.5,	7,	85
60	72.8	96,	101,	110,	110,	26,	27,	29,	34,	4,	5.5,	70
65	73.9	69,	74,	88,	97,	35,	37,	39,	39,	9.5,	11,	50
70	77.8	67,	68,	71,	89,	28,	36,	41,	49,	6.5,	7.5,	50
75	82.5	69,	77,	77,	89,	33,	36,	38,	48,	9.5,	9.5,	50
85	76.0	80,	89,	93,	93,	43,	46,	55,	56,	13,	16,	25
95	82.1	91,	95,	101,	112,	44,	53,	58,	67,	12,	15,	25
105	75.5	95,	96,	98,	98,	67,	73,	87,	94,	11,	20,	10
115	88.6	93,	97,	99,	109,	90,	90,	99,	109,	19,	23,	10
125	85.3	95,	96,	101,	102,	82,	83,	84,	97,	37,	39,	5
135	96.9	94,	95,	98,	98,	97,	97,	106,	107,	99,	106,	<1
140b	98.3	101,	101,	102,	105,	94,	103,	105,	105,	82,	87,	<1

Day 140. 50 ml. of suspension removed to start a further one (Table 29b,). 2.5 ml. of 1% 2,4-D solution and distilled water added to make suspension 250 ml. of 100 ppm. Shaken and sampled.

140a	98.3	71,	72,	76,	78,	15,	15,	18,	20,	3,	3,	130
150	91.7	84,	84,	85,	89,	32,	32,	35,	46,	5.5,	6.5,	70
160	100.1	93,	94,	94,	98,	40,	42,	50,	61,	5,	7,	55
170	96.6	87,	91,	92,	97,	54,	72,	74,	78,	7,	7,	15
190	100.1	98,	99,	103,	110,	90,	90,	91,	92,	23,	25,	9
210	97.1	95,	96,	96,	102,	87,	93,	95,	102,	42,	54,	3
220	99.8	97,	101,	104,	107,	104,	106,	108,	109,	82,	86	<1

Day 225. 2.5 ml. of 1% 2,4-D solution added to makecthe suspension approximately 250 ml. of 100 ppm. 2,4-D. Shaken and sampled.

225a	96.4	85,	87,	90,	95,	18,	19,	21,	24,	3,	4,	100
230	99.1	85,	86,	86,	89,	25,	27,	27,	38,	4,	5,	80

Table 29b. Breakdown of 2,4-D in soil-less suspension.

Key to columns in table: as in Table 29a, above.

Incubation started on 5/6/53. 200 ml. of distilled water and 2.5 ml. of 1% 2,4-D solution were boiled together for 30 mins. After cooling, this solution was inoculated with 50 ml. of clear liquid from an"active" suspension (Table 29a,). The 250 ml. of 100 ppm. 2,4-D suspension was then shaken and sampled, and then incubated at 28°C.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
0	98.3	78, 79, 81, 90,	17, 18, 20, 23,	3, 4,	100
10	91.7	92,101,103,106,	36, 37, 41, 46,	5.5,6.5,	55
20	100.1	97,104,105,114,	70, 75, 79, 82,	9, 11,	20
30	96.6	94, 95,100,101,	94, 98,103,112,	27, 33,	7
50	100.1	101,103,106,106,	96, 98,101,106,	100,104,	<1

Day 55. 2.5 ml. of 1% 2,4-D solution added to the suspension and water to make a total of 250 ml. of 100 ppm. 2,4-D. Shaken, sampled and re-incubated.

55a	87.7	96, 97,105,105,	26, 31, 32, 39,	4.5,5.5,	65
60	97.1	84, 84, 89, 90,	27, 27, 29, 31,	4, 4,	75
70	99.8	95,103,105,107,	31, 31, 33, 34,	4, 5,	70
80	97.0	86, 89, 90, 97,	27, 27, 28, 35,	4, 5,	80

Table 29c. Transfer of 2,4-D adaptation to crushed, sterile pot.

Key to columns in table: as in Table 29.

Perfusion started on 22/7/52 with 50 gm. of 2 to 4 mm., sterilised, dried, acid-washed, crushed flower-pot and 250 ml. of 100 ppm. 2,4-D solution prepared from the "active" perfusate drained from a 2,4-D enriched perfuser (Table 42f,) on 22/7/52. First sample taken after 1 hrs. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	46.8	85,	90,	98,	103,	21,	21,	24,	26,	4.5,	4.5,	90
3	61.9	81,	89,	95,	102,	19,	20,	21,	24,	3,	5,	85
6	61.9	76,	78,	81,	115,	19,	20,	21,	24,	3,	5,	85
15	63.3	82,	82,	89,	92,	84,	89,	90,	100,	97,	104,	<1
17b	62.8	96,	97,	104,	120,	84,	94,	100,	100,	96,	96,	<1
17a	62.8	73,	73,	75,	81,	17,	19,	19,	21,	3,	5,	120
19	50.9	63,	65,	71,	79,	16,	18,	26,	37,	4,	4,	110
21	61.4	68,	86,	90,	96,	21,	21,	23,	28,	5,	6.5,	110
23	57.2	80,	93,	96,	107,	26,	26,	28,	33,	5,	11,	60
25	61.9	96,	97,	102,	110,	58,	60,	63,	74,	39,	39,	5
27b	53.9	84,	85,	89,	91,	100,	106,	106,	108,	100,	108,	<1
27a	53.9	72,	76,	76,	87,	18,	19,	20,	20,	3.5,	5.5,	120
29	67.3	57,	59,	61,	71,	15,	16,	19,	20,	4.5,	4.5,	115
31	49.6	75,	75,	77,	91,	24,	30,	30,	32,	4,	8,	70
33	57.1	105,	109,	110,	110,	100,	109,	110,	114,	86,	86,	1
35b	49.7	87,	97,	99,	113,	91,	93,	93,	103,	75,	83,	1
35a	49.7	69,	71,	76,	101,	20,	22,	24,	26,	4,	6,	95
37	43.4	78,	81,	88,	92,	23,	25,	25,	30,	4.5,	7,	85
39	60.0	63,	67,	75,	85,	28,	33,	38,	53,	6.5,	8.5,	50
41	73.6	95,	98,	105,	109,	91,	91,	97,	98,	83,	91,	<1
43b	72.0	100,	101,	106,	108,	106,	108,	110,	121,	88,	93,	<1
43a	72.0	71,	72,	72,	81,	15,	19,	20,	25,	3,	4,	120
45	57.9	52,	55,	57,	62,	19,	21,	22,	29,	1.5,	5.0,	110

Day 17. A little water and 2.5 ml. of 1% 2,4-D solution added to the perfusate, making it approximately 250 ml. of 100 ppm. 2,4-D. Sampled after 1 hr. perfusion.

Day 27. A little water and 2.5 ml. of 1% 2,4-D solution added to the perfusate, making it approximately 250 ml. of 100 ppm. 2,4-D. Sampled after 1 hr. perfusion.

Day 35. A little water and 2.5 ml. of 1% 2,4-D solution added to the perfusate, making it approximately 250 ml. of 100 ppm. 2,4-D. Sampled after 1 hr. perfusion.

Day 43. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

Table 29c. continued.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
47	59.6	47, 50, 60, 72,	17, 18, 23, 23,	3.5,3.5,	100
49	71.8	67, 92, 95,100,	26, 29, 32, 33,	7,9.5,	55
51	61.2	80, 85, 93,101,	80, 90, 93,110,	46, 82,	2.5
53	51.8	71, 75, 97,100,	66, 75, 89,100,	79, 85,	1
55b	52.0	96, 98,100,115,	104,104,117,117,	119,121,	<1

Day 55. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

55a	49.2	53, 59, 69, 81,	24, 24, 26, 31,	4, 4,	120
57	52.0	58, 62, 67, 69,	15, 19, 19, 23,	4, 6,	120
59	52.8	47, 51, 57, 64,	21, 23, 23, 23,	4,5.5,	100
61	53.3	75, 90, 94,105,	32, 32, 40, 41,	11, 15,	60
65	49.0	92, 96,100,108,	82, 82, 86, 88,	75, 77,	1

Day 66. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

66a	49.0	63, 67, 69, 73,	27, 27, 29, 31,	6, 8,	80
68	51.2	68, 70, 70, 78,	29, 33, 33, 33,	4, 4,	80
71	56.7	58, 60, 65, 67,	30, 32, 34, 34,	9, 13,	55
74	63.5	82, 85, 87, 90,	95, 95,101,104,	58, 87,	15
77	74.3	78, 86, 96,107,	70, 71, 78, 81,	55, 57,	2.5
79b	76.5	97, 99,110,111,	98, 99, 99,102,	92,101,	<1

Day 79. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

79a	76.5	68, 68, 85, 85,	17, 19, 26, 29,	2.5, 4,	100
82	61.6	52, 55, 57, 62,	18, 23, 23, 24,	5, 5,	95
91b	75.0	93, 99,109,109,	107,109,113,117,	112,113,	<1

Day 91. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

91a	75.0	64, 76, 89, 91,	23, 24, 24, 27,	4,5.5,	95
94	76.4	75, 76, 80, 89,	18, 19, 24, 33,	4, 5,	90
97	77.5	76, 78, 89, 96,	83, 84, 97,102,	30, 36,	10
100b	60.3	96, 98,116,116,	100,101,108,115,	118,121,	<1

Day 100. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

100a	72.2	62, 64, 66, 68,	18, 18, 20, 24,	3, 4,	130
103	67.8	77, 83, 83, 88,	17, 21, 21, 22,	4.5,4.5,	105
106	66.0	82, 88, 94,103,	47, 48, 55, 55,	10, 17,	20
109	65.0	82, 91, 92,100,	109,119,119,122,	103,108,	<1
112b	68.8	103,105,113,131,	97, 97,106,108,	96, 97,	<1

Day 112. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

Table 29c. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
112a	68.8	84,	84,	97,	100,	19,	20,	26,	28,	1.5,	4.5,	95
115	69.8	63,	70,	72,	79,	20,	21,	29,	29,	4.5,	5.5,	70
118	73.1	55,	60,	67,	67,	22,	23,	23,	26,	4,	7,	70
121	72.5	83,	84,	85,	88,	29,	29,	30,	37,	5.5,	8.5,	70
124	76.2	84,	88,	89,	96,	25,	26,	28,	28,	6.5,	6.5,	75
127	83.0	82,	86,	88,	92,	30,	30,	34,	41,	2.5,	6,	70
Day 127a. 5 ml. of 10% $(NH_4)H_2PO_4$ solution added to the perfusate without draining.												
130	66.0	86,	95,	99,	102,	77,	79,	83,	85,	26,	30,	10
133	58.3	84,	88,	93,	107,	88,	94,	107,	110,	91,	91,	1
Day 136. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.												
136a	62.9	65,	70,	78,	84,	21,	21,	22,	24,	5,	5,	110
139	81.6	62,	66,	67,	84,	18,	19,	21,	24,	3.5,	5,	120
142	85.1	93,	96,	98,	105,	92,	92,	95,	99,	106,	111,	<1
145	65.0	98,	103,	109,	115,	92,	100,	108,	109,	112,	120,	<1
148	77.1	82,	83,	86,	88,	83,	86,	86,	87,	101,	109,	<1
172b	73.3	86,	87,	90,	102,	90,	93,	94,	94,	84,	94,	<1
Day 172. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 0.2% $(NH_4)H_2PO_4$. Perfuser re-started, sampled after 1 hr. perfusion.												
172a	73.3	57,	59,	59,	65,	26,	27,	29,	29,	4,	5.5,	70
175	65.3	58,	58,	63,	72,	21,	24,	26,	34,	3,	3,	85
178	82.8	42,	50,	50,	56,	17,	19,	20,	23,	7.5,	8.5,	115
181	67.5	101,	106,	106,	114,	78,	87,	99,	109,	63,	68,	1
184	78.5	64,	70,	95,	112,	85,	89,	95,	95,	80,	80,	<1
Day 187. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 0.2% $(NH_4)H_2PO_4$. Perfuser re-started, sampled after 1 hr. perfusion.												
187a	73.5	60,	78,	82,	90,	18,	22,	22,	26,	2,	4,	110
190	84.3	68,	75,	76,	79,	16,	19,	24,	27,	1,	2.5,	105
193	76.6	61,	72,	73,	81,	17,	18,	19,	20,	5,	5,	120
196	87.1	84,	84,	92,	97,	38,	41,	50,	54,	11,	13,	40
199	91.0	82,	87,	90,	93,	70,	76,	79,	95,	87,	105,	<1
202b	84.2	83,	89,	90,	94,	65,	72,	74,	100,	90,	110,	<1
Day 202. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.												
202a	84.2	52,	55,	56,	66,	14,	14,	15,	19,	2.5,	2.5,	180
205	78.8	65,	71,	74,	77,	19,	20,	23,	24,	4,	4,	110
208b	77.4	80,	81,	86,	94,	86,	90,	94,	113,	71,	75,	1.5

Table 29c. continued.

Day 208. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-D. solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
208a	77.4	30, 32, 32, 35,	14, 15, 16, 17,	2.5, 4,	170
211	82.6	85, 88, 98,108,	27, 28, 28, 42,	8.5, 11,	80
214	83.3	68, 80, 81, 87,	90, 92, 99,101,	93,102,	<1
217b	80.8	84, 85, 92, 92,	88,103,103,104,	64, 69,	2

Day 217. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

217a	80.8	46, 47, 59, 71,	16, 16, 21, 22,	2.5,2.5,	160
220	94.5	54, 59, 59, 64,	17, 18, 19, 23,	1, 3,	140
223	85.3	61, 68, 70, 88,	15, 17, 19, 19,	3.5,4.5,	130
226	83.5	87, 89, 95,108,	37, 38, 39, 49,	8.5,9.5,	45
229	76.0	71, 96, 99,100,	92, 95, 97,103,	84, 99,	<1
232b	92.9	73, 75, 83, 90,	97,103,104,117,	85, 86,	<1

Day 232. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

232a	88.4	48, 55, 59, 77,	16, 20, 20, 26,	3.5,4.5,	115
235	84.1	80, 88, 88,102,	20, 26, 26, 32,	4, 6,	80
238	72.8	75,100,107,111,	40, 40, 48, 49,	8,9.5,	35
241	71.1	69, 77, 79, 80,	66, 67, 79, 91,	45, 48,	4
244	73.9	87, 89, 99,106,	99,103,106,111,	110,111,	<1
247	79.9	86, 91,100,110,	88, 88, 90,104,	94, 95,	<1

Table 30. Direct perfusion of 2,4-D, adaptation retained through perfusion with water only.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Indicated 2,4-D concentration in the perfusate (ppm.).

Perfusion started on 14/6/51 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried June 1951,) and 250 ml. of 1,000 ppm. 2,4-D solution, (common solution used for this perfuser and those of Tables 30d, 30e, and 30f,). No sample taken.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
18	43.7	69,	71,	82,	85,	34,	37,	37,	48,	4.5,	7,	550
21	44.4	70,	79,	84,	106,	36,	45,	47,	47,	7,	7,	350
23	40.7	86,	86,	89,	91,	89,	94,	98,	108,	89,	96,	<1

Day 27. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

27a	36,7	68,	68,	68,	68,	44,	52,	55,	57,	5.5,	8,	450
29	35.2	102,	108,	111,	119,	54,	57,	57,	71,	8.5,	17,	250
31	42.8	91,	91,	91,	94,	61,	80,	87,	96,	47,	68,	25

Day 35. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

35a.	37.6	69,	72,	72,	72,	48,	48,	51,	53,	5.5,	5.5,	600
38	37.4	89,	91,	99,	112,	72,	75,	78,	83,	14,	24,	130
40	33.5	87,	99,	110,	114,	99,	99,	102,	114,	90,	90,	<10

Day 43. Perfuser drained and refilled with 250 ml. of distilled water. Perfuser re-started and allowed to continue, with the water only, for 60 days.
Day 103. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

103	54.1	68,	72,	81,	87,	26,	26,	30,	30,	5.5,	7.5,	75
106	54.1	76,	76,	79,	83,	41,	42,	42,	46,	7.5,	17,	40,
109	58.4	104,	118,	120,	123,	99,	101,	101,	139,	132,	152,	<1

Day 112. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

112a	74.3	75,	77,	85,	94,	16,	16,	22,	23,	2.5,	2.5,	1,150
116	62.1	58,	63,	68,	77,	24,	27,	29,	32,	3,	3,	1,000
118	72.4	61,	65,	87,	94,	18,	21,	29,	29,	4,	5.5,	950

Table 30. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
120	67.4	74,	77,	80,	108,	22,	22,	24,	37,	7.5,	7.5,	750
122	67.7	71,	77,	78,	84,	36,	37,	40,	52,	7.5,	14,	500
124	57.4	78,	85,	110,	114,	58,	66,	75,	91,	23,	31,	100
126	56.5	59,	82,	89,	97,	92,	97,	110,	113,	74,	94,	10
Day 128. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.												
128a	43.4	71,	71,	90,	106,	25,	25,	28,	28,	7,	7,	850
131	51.7	72,	79,	83,	97,	25,	25,	27,	33,	6,	7.5,	850
133	53.3	90,	96,	104,	111,	30,	36,	40,	43,	4,	7.5,	550
135	52.3	82,	84,	88,	90,	29,	33,	40,	50,	4,	9.5,	500
137	47.7	74,	76,	78,	94,	34,	38,	40,	44,	8.5,	13,	400
139	72.3	80,	87,	91,	104,	39,	52,	52,	66,	6,	13,	300
141	66.1	85,	100,	104,	118,	52,	67,	67,	76,	6,	9,	200
143	55.8	54,	77,	90,	92,	66,	72,	74,	84,	27,	36,	80
Day 145. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.												
145a	71.5	67,	69,	69,	90,	17,	20,	32,	39,	4,	4,	850
148	70.7	65,	78,	91,	98,	23,	24,	31,	33,	4,	4,	800
150	62.8	73,	84,	86,	110,	26,	27,	27,	43,	3,	4.5,	800
152	75.2	75,	89,	93,	101,	23,	24,	35,	37,	4,	5.5,	700
154	79.1	69,	71,	76,	81,	22,	22,	24,	27,	2.5,	4,	1,000
156	75.2	84,	84,	89,	102,	37,	39,	39,	41,	4,	8,	500
158	81.6	85,	86,	91,	93,	20,	21,	22,	26,	5,	7.5,	800
160	69.5	89,	101,	101,	106,	27,	27,	29,	30,	6,	6,	750
162	59.4	94,	99,	116,	120,	30,	35,	42,	76,	5,	6.5,	550
164	55.9	129,	131,	138,	140,	52,	57,	63,	66,	14,	16,	200
166	68.3	95,	100,	101,	103,	44,	44,	72,	72,	13,	15,	250
168	66.0	95,	104,	108,	111,	60,	83,	86,	92,	111,	128,	<10
170b	67.4	108,	111,	116,	129,	86,	86,	88,	140,	112,	134,	<10
Day 170. Perfuser not drained. 25 ml. of 1% 2,4-D solution added, making the perfusate approximately 250 ml. of approx. 100 ppm. 2,4-D. Perfuser sampled after 1 hr. perfusion.												
170a	58.6	77,	77,	82,	87,	26,	31,	32,	34,	3.5,	5,	650
173	67.6	55,	86,	89,	105,	24,	25,	30,	46,	4.5,	14,	600
175	70.7	65,	78,	81,	82,	28,	30,	34,	35,	3,	4,	650
178	70.3	77,	79,	83,	89,	20,	21,	21,	24,	4,	6,	1,000
181	72.4	79,	80,	86,	91,	19,	22,	25,	30,	4,	4,	950
184	66.9	69,	76,	78,	91,	18,	22,	25,	40,	7.5,	7.5,	1,100

Table 30d. Direct perfusion of 2,4-D; adaptation retained through perfusion with water only.

Key to columns in table: as in Table 30.
Details of perfusion set-up: as in Table 30.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0		No initial sample taken.										
18	43.7	69,	80,	85,	96,	80,	85,	87,	92,	21,	23,	100
21	44.4	99,	104,	106,	108,	72,	90,	90,	106,	95,	97,	<10

Day 23. Perfuser drained and refilled with 250 ml. of 500 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

23a	40.7	66,	66,	71,	71,	44,	47,	52,	54,	5,	7.5,	500
25	37.7	88,	93,	112,	125,	61,	64,	66,	77,	11,	16,	200
27	36.7	93,	93,	93,	95,	76,	76,	87,	93,	63,	65,	20
29	35.2	77,	80,	83,	83,	91,	97,	105,	111,	94,	105,	10

Day 34. Perfuser drained and refilled with 250 ml. of 500 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

34a	34.8	75,	78,	81,	92,	43,	43,	46,	58,	8.5,	8.5,	400
37	36.6	90,	93,	107,	109,	68,	68,	77,	79,	22,	22,	100
39	30.9	84,	84,	88,	97,	97,	104,	113,	120,	113,	130,	<10

Day 43. Perfuser drained and refilled with 250 ml. of distilled water. Perfusion re-started and allowed to go on for 30 days unchanged.
Day 73. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

73a	67.2	77,	80,	85,	88,	28,	30,	30,	42,	4.5,	9,	70
75	72.8	80,	88,	94,	99,	26,	30,	32,	34,	4,	8,	65
77	62.6	75,	78,	82,	112,	30,	30,	30,	32,	6.5,	11,	65
79b	58.6	95,	99,	104,	111,	38,	41,	46,	48,	10,	12,	35

Day 79. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

79a	58.6	84,	85,	85,	95,	20,	20,	22,	22,	3,	5,	1,000
83	61.7	81,	106,	106,	117,	26,	34,	41,	47,	3,	5,	600
85	51.8	89,	93,	97,	100,	29,	31,	33,	35,	8,	12,	550
87	42.3	109,	111,	125,	149,	54,	64,	64,	64,	7,	7,	350
89	70.5	99,	99,	112,	114,	50,	54,	55,	60,	10,	13,	250
91	46.2	89,	102,	106,	126,	106,	106,	108,	117,	82,	87,	10

Day 94. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

94a	62.6	69,	77,	83,	93,	22,	26,	26,	30,	3,	6.5,	900
98	76.3	72,	79,	84,	105,	14,	16,	18,	21,	4,	5,	950

Table 30d. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
100	68.8	74,	80,	83,	89,	19,	29,	36,	36,	4.5,	4.5,	800
102	48.5	66,	70,	72,	77,	29,	31,	33,	35,	4,	6,	700
104	69.3	91,	94,	101,	107,	22,	22,	25,	40,	4.5,	4.5,	750
106	46.0	87,	87,	91,	104,	33,	33,	37,	52,	4.5,	4.5,	750
108	72.3	71,	87,	89,	98,	26,	29,	32,	47,	7,	7,	600
110	54.9	57,	67,	69,	80,	33,	35,	36,	55,	7.5,	15,	500
112	74.3	80,	82,	84,	92,	53,	53,	54,	69,	9.5,	15,	300
114	72.9	85,	85,	91,	98,	77,	84,	89,	110,	92,	102,	<10

Day 116. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

116a	62.1	55,	55,	56,	58,	19,	23,	23,	24,	3,	8,	1,000
121	59.3	61,	64,	76,	108,	22,	22,	29,	32,	5,	6.5,	1,000
123	67.7	67,	67,	70,	71,	16,	22,	22,	30,	6,	7.5,	1,000
125	69.9	67,	79,	89,	103,	16,	17,	17,	19,	3,	4,	1,000
127	36.5	71,	82,	91,	102,	36,	36,	36,	52,	5.5,	5.5,	650
129	60.9	51,	61,	67,	72,	25,	30,	31,	40,	5,	6.5,	650
131	51.7	52,	60,	83,	120,	21,	21,	23,	25,	4,	6,	950
133	53.3	45,	57,	64,	64,	19,	21,	21,	23,	5.5,	5.5,	1,100
135	52.3	84,	92,	98,	109,	27,	27,	29,	33,	4,	8,	800
137	47.7	80,	97,	99,	99,	29,	29,	32,	42,	4,	4,	750
139	72.3	71,	86,	96,	100,	21,	24,	29,	32,	4,	4,	850
141	66.1	77,	77,	88,	92,	25,	28,	29,	32,	3,	4.5,	800
143	55.8	92,	99,	102,	113,	32,	36,	36,	38,	3.5,	5.5,	550
145	66.4	69,	78,	80,	101,	23,	23,	23,	29,	4.5,	4.5,	950
147	64.4	73,	81,	86,	87,	22,	23,	25,	31,	3,	4.5,	950
149	68.3	56,	60,	67,	112,	19,	23,	25,	31,	3,	4.5,	950
151	67.0	72,	75,	78,	84,	25,	30,	31,	42,	6,	7.5,	650
153	74.7	85,	85,	91,	93,	20,	21,	29,	32,	4,	4,	850
155	75.2	76,	77,	81,	91,	25,	28,	31,	35,	4,	8,	700
157	72.9	69,	69,	85,	88,	19,	19,	23,	30,	7,	8.5,	950
159	78.6	89,	91,	94,	103,	19,	29,	31,	32,	4,	5,	700
161	62.2	82,	89,	94,	104,	26,	26,	39,	45,	5,	6.5,	600
163	64.1	53,	70,	70,	75,	30,	30,	33,	34,	4.5,	8,	600
165	52.7	76,	91,	108,	118,	30,	36,	38,	46,	5.5,	7.5,	500
167	59.7	80,	82,	108,	127,	30,	30,	32,	45,	8.5,	12,	500
169	61.6	78,	81,	85,	98,	55,	59,	60,	62,	8,	13,	250
171	58.6	77,	91,	91,	132,	50,	55,	63,	96,	17,	19,	150
174	70.7	88,	89,	92,	93,	82,	82,	98,	99,	52,	67,	25
176	76.3	82,	102,	106,	109,	76,	88,	89,	92,	58,	66,	20,

Day 177. Perfuser not drained, but 25 ml. of 2,000 ppm. 2,4-DCP. solution added, to make perfusate approximately 250 ml. of 200 ppm. 2,4-DCP. Sampled ~~before adding and~~ after 1 hr.

Day.	177a,	178,	179,	180,	181,	182,	183,	184,	185,	186,	187,
Col.Reading.	79,	47,	44.4,	43.4,	37,	36.8,	36,	32.2,	29,	27.4,	28,
PPM.2,4-DCP.	158,	94,	89,	87,	74,	73.5,	72,	64.5,	58,	55,	56,

Table 30e. Direct perfusion of 2,4-D; adaptation retained through perfusion with water only.

Key to columns in table: as in Table 30.
Details of perfusion set-up: as in Table 30.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0		No initial sample taken.										
18	43.7	87,	87,	94,	110,	50,	53,	53,	55,	7,	7,	450
21	44.4	81,	84,	86,	99,	43,	43,	45,	47,	7,	9,	400
23	40.7	94,	101,	106,	111,	96,	101,	111,	120,	94,	113,	<10

Day 27. Perfuser drained and refilled with 250 ml. of 500 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr:

27a	36.7	79,	82,	87,	87,	44,	49,	52,	55,	8,	11,	300
29	35.2	77,	80,	84,	84,	51,	51,	54,	57,	8.5,	8.5,	300
31	42.8	84,	87,	91,	96,	58,	63,	63,	73,	12,	19,	200
33	34.8	81,	98,	112,	112,	95,	95,	104,	109,	86,	129,	<10

Day 38. Perfuser drained and refilled with 250 ml. of 500 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

38	30.9	84,	87,	91,	97,	52,	52,	58,	68,	9.5,	26,	300
40	33.5	104,	107,	111,	114,	57,	60,	63,	63,	6,	9,	350
42	38.4	89,	91,	94,	114,	47,	55,	57,	60,	5,	8,	300
44	33.6	82,	86,	98,	107,	60,	63,	75,	75,	12,	15,	200
46	41.3	78,	80,	80,	83,	61,	63,	68,	73,	24,	24,	90
48	42.8	94,	94,	96,	98,	77,	87,	98,	100,	77,	87,	10

Day 51. Perfuser drained and refilled with 250 ml. of distilled water. Perfusion re-started and allowed to go on for 50 days unchanged.
Day 101. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

101a	57.5	59,	68,	103,	145,	21,	21,	26,	28,	3.5,	5,	100
104	69.3	93,	106,	111,	120,	23,	29,	30,	32,	3,	6,	75
107	46.0	63,	76,	87,	120,	91,	98,	100,	102,	102,	106,	<1
109	58.4	67,	77,	79,	84,	99,	101,	112,	118,	104,	108,	<1
111	67.6	85,	86,	87,	105,	89,	96,	105,	126,	56,	90,	1.5

Day 112. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

112a	74.3	74,	84,	88,	97,	16,	18,	20,	20,	2.5,	4,	1,100
116	62.1	66,	76,	82,	102,	24,	24,	26,	27,	3.5,	5,	900
118	72.4	69,	73,	79,	94,	23,	25,	35,	35,	3,	4,	900
120	67.4	72,	75,	76,	90,	18,	18,	19,	22,	3,	4.5,	1,000
122	67.7	56,	62,	72,	96,	25,	28,	33,	37,	4.5,	9,	700
124	57.4	78,	82,	84,	108,	33,	33,	42,	47,	3.5,	5,	600
126	56.5	94,	94,	97,	106,	39,	44,	46,	57,	5.5,	9,	350
128	43.4	83,	99,	106,	118,	53,	58,	60,	74,	7,	16,	200
130	44.2	107,	136,	141,	154,	91,	117,	118,	124,	100,	117,	<10
132	43.2	95,	95,	100,	123,	74,	90,	97,	111,	84,	116,	<10

Table 30e. continued.

Day 134. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
134a	43.1	81,	100,	112,	114,	30,	30,	33,	44,	4.5,	7,	650
137	47.7	80,	80,	84,	90,	27,	29,	29,	43,	6.5,	11,	700
139	72.3	73,	75,	89,	93,	24,	25,	30,	33,	4,	4,	750
141	66.1	65,	65,	74,	74,	20,	28,	29,	37,	3,	4.5,	750
143	55.8	83,	84,	94,	112,	25,	25,	29,	36,	3.5,	5.5,	800
145	66.4	81,	90,	93,	98,	21,	24,	26,	42,	3,	4.5,	900
147	64.4	81,	90,	99,	101,	26,	28,	30,	36,	3,	4.5,	800
149	68.3	64,	65,	76,	92,	17,	30,	31,	34,	4.5,	4.5,	750
151	67.0	72,	72,	91,	96,	30,	31,	33,	35,	4.5,	6,	650
153	74.7	65,	67,	91,	95,	19,	19,	21,	23,	4,	5.5,	900
155	75.2	55,	63,	65,	75,	27,	32,	33,	37,	3,	4,	750
157	72.9	67,	72,	82,	99,	14,	21,	25,	26,	2.5,	5.5,	900
159	78.6	79,	82,	96,	110,	24,	27,	33,	50,	4,	5,	700
161	62.2	56,	71,	81,	104,	27,	31,	35,	50,	5,	6.5,	600
163	64.1	69,	75,	75,	95,	22,	23,	25,	30,	4.5,	4.5,	650
165	52.7	99,	104,	120,	120,	44,	55,	61,	72,	5.5,	5.5,	300
167	59.7	107,	110,	117,	133,	42,	67,	67,	77,	10,	14,	200
169	61.6	117,	124,	125,	127,	60,	67,	73,	76,	11,	15,	200
171	58.6	109,	121,	123,	132,	87,	89,	91,	111,	21,	22,	100
173	67.6	89,	95,	99,	105,	86,	90,	90,	105,	92,	96,	<10

Day 174. Perfuser not drained. 10 ml. of 2,000 ppm. 2,4-DCP solution added to make the perfusate approximately 250 ml. of 80 ppm. 2,4-DCP. Sampled after 1 hr. perfusion.

Day.	174a1hr.,	174a2hr.,	175b,
Colourimeter Reading.	28.5 ,	23.0 ,	1.7,
PPM. 2,4-DCP. in perfusate.	57 ,	46 ,	3.5,

Day 175. 10 ml. of 2,000 ppm. 2,4-DCP solution added to the perfuser making it approximately 250 ml. of 80 ppm. 2,4-DCP. Sampled after 1 hr. perfusion.

Day.	175a1hr.,	176 ,	177b ,
Colourimeter Reading.	26.6 ,	7.1,	2.9,
PPM. 2,4-DCP in perfusate.	53 ,	14 ,	6 ,

Day 177. 10 ml. of 2,000 ppm. 2,4-DCP solution added to the perfuser making it approximately 250 ml. of 80 ppm. 2,4-DCP. Sampled after 5 hr. perfusion.

Day.	177a5hr,	178,	179,	180,	181,	182,	183,	184,	185,
Col.Read.	24.2 ,	17.4,	13 ,	15 ,	12.2,	9.8,	11,	8.5,	8.3,
PPM.2,4-DCP.	48.5 ,	35 ,	26 ,	30 ,	24.5,	19.5,	22,	17,	16.5,

Table 30f. Direct perfusion of 2,4-D; adaptation retained through perfusion with water only.

Key to columns in table: as in Table 30.
Details of perfusion set-up: as in Table 30.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
0		No initial sample taken.			
18	43.7	101,108,108,121,	103,114,119,124,	117,121,	<10
Day 22. Perfuser drained and refilled with 250 ml. of 500 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.					
22a	40.6	79, 81, 81, 86,	44, 44, 49, 59,	5, 5,	500
24	37.7	88, 88, 90, 96,	43, 43, 48, 53,	8, 16,	400
26	37.2	89, 89, 94,102,	54, 57, 59, 65,	11, 11,	250
28	35.2	77, 80, 85, 85,	85, 97,102,108,	80,100,	<10
Day 33. Perfuser drained and refilled with 250 ml. of 500 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.					
33	34.8	95, 98,104,118,	49, 49, 52, 52,	8.5, 17,	300
36	34.6	113,113,113,118,	64, 64, 64, 75,	20, 23,	100
38	37.4	99,102,104,107,	102,107,107,137,	107,110,	<10
Day 41. Perfuser drained and refilled with 250 ml. of distilled water. Perfusion re-started and allowed to go on for 12 days unchanged.					
Day 53. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.					
53	44.9	83, 87, 92, 92,	31, 33, 33, 45,	4.5,6.5,	65
54	43.5	83, 85, 97,104,	22, 28, 28, 37,	4.5,4.5,	75
55	74.8	84, 88, 88,103,	19, 23, 26, 27,	4,5.5,	80
56	64.2	97,100,103,109,	28, 30, 31, 34,	6, 6,	60
57	64.2	81, 81,101,112,	30, 33, 33, 36,	6, 6,	60
58	64.2	86,104,117,120,	109,110,112,121,	72, 97,	<1
Day 60. Perfuser drained and refilled with 150 ml. of 500 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.					
60a	45.2	100,122,124,133,	31, 33, 35, 51,	6.5, 11,	600
63	48.9	94,102.117,129,	29, 29, 29, 31,	8, 10,	500
65	67.3	74, 94, 94,104,	25, 27, 28, 28,	6,7.5,	600
66b	64.1	84, 84, 87,100,	27, 28, 39, 60,	4.5, 8,	550
Day 66. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.					
66a	64.1	64, 70, 70, 78,	20, 20, 22, 22,	4.5,4.5,	1,100
69	58.7	89, 94, 96,103,	36, 36, 41, 56,	5, 7,	550
71	70.0	83, 83, 87, 89,	24, 29, 34, 50,	4, 4,	800
73	67.2	79, 79, 79, 91,	30, 30, 31, 42,	4.5,4.5,	700

Table 30f. continued.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
75	72.8	81, 87, 87,103,	25, 26, 32, 36,	5.5,5.5,	750
77	62.6	93, 95,102,107,	34, 35, 42, 58,	6.5, 8,	550
79	58.6	82, 94,102,104,	39, 41, 43, 46,	6.0, 10,	450
81	60.1	110,110,115,118,	42, 43, 58, 68,	12, 12,	300
83	61.7	97, 97,104,135,	73, 73, 73, 75,	13, 15,	150
85	51.8	106,112,139,139,	83, 83, 85,114,	102,112,	<10

Day 87. Perfuser drained and refilled with 250 ml. of 500 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

87a	42.3	109,111,120,132,	28, 31, 38, 38,	4.5, 7,	600
91	46.2	63, 74, 91, 93,	28, 30, 37, 41,	4.5,6.5,	600
93	49.2	98,110,114,128,	39, 39, 45, 47,	8, 8,	450
95	62.6	96, 96,104,109,	34, 35, 38, 45,	5, 5,	550
97	66.5	54, 56, 56, 62,	33, 35, 36, 42,	6, 9,	550
99	49.3	87, 87, 93,136,	59, 71, 81, 87,	14, 14,	200
101	57.5	90,106,110,118,	82, 90,101,103,	31, 52,	50
103	69.3	45, 45, 48, 48,	65, 68, 70, 81,	62, 71,	20
105	73.0	96, 97,107,138,	96,100,106,108,	88, 92,	<10

Day 106. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D. Perfuser re-started, sampled after 1 hr.

106a	46.0	85, 85, 89,100,	33, 37, 37, 41,	6.5,8.5,	550
110	54.9	62, 66, 71, 86,	20, 22, 22, 38,	3.5,7.5,	1,000
112	69.1	71, 78, 78, 94,	26, 28, 28, 35,	4.5, 6,	750
114	72.9	106,108,110,113,	95, 99,103,120,	72,108,	<10

Day 116. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

116a	62.1	58, 64, 77, 84,	24, 26, 26, 32,	3, 8,	900
121	59.3	56, 64, 69, 73,	24, 25, 25, 27,	5,6.5,	900
123	67.7	59, 77, 77, 80,	22, 25, 25, 27,	4.5,4.5,	900
125	69.9	66, 84, 89, 93,	20, 29, 29, 30,	4, 6,	750
127	36.5	69, 71, 74, 82,	44, 47, 47, 52,	8, 11,	350
129	60.9	62, 67, 71, 86,	30, 31, 36, 51,	6.5, 8,	500
131	51.7	77, 77, 85, 91,	74, 74, 76, 87,	21, 25,	100
133	53.3	88, 96,115,115,	102,102,104,132,	70,105,	<10

Day 136. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

136a	49.8	76, 82, 86, 92,	22, 22, 24, 26,	6, 6,	900
140	60.7	66, 71, 73, 89,	30, 30, 33, 36,	5, 5,	700
142	66.8	76, 78, 82, 94,	27, 28, 30, 37,	4.5,4.5,	700
144	63.8	61, 80, 86,128,	28, 33, 36, 42,	4.5,9.5,	600
146	71.5	91, 97, 98, 99,	22, 24, 25, 42,	4, 4,	750
148	70.7	69, 72, 77, 86,	27, 27, 33, 40,	4,5.5,	700
150	62.8	94,102,107,111,	22, 22, 29, 30,	4.5,7.5,	750

Table 30f. continued.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
152	75.2	79, 79, 96,101,	27, 28, 35, 47,	5.5, 7,	550
154	79.1	59, 64, 69, 92,	28, 28, 29, 36,	2.5, 4,	750
156	75.2	72, 75, 87, 89,	28, 33, 36, 43,	7, 8,	600
158	81.6	52, 52, 54, 65,	27, 32, 33, 41,	5, 9,	650
160	69.5	89, 92, 96,108,	37, 37, 46, 50,	6,8.5,	500
162	59.4	64, 67, 74, 78,	35, 38, 40, 42,	8.5, 12,	500
164	55.9	52, 61, 77, 79,	32, 34, 38, 45,	11, 11,	400
166	68.3	60, 83,101,114,	32, 34, 35, 59,	6, 10,	400
168	66.0	68, 75, 77, 86,	51, 56, 66, 71,	12, 14,	200
170	67.4	89, 94,107,119,	77, 88, 97,119,	19, 19,	100
172	60.0	88, 95,120,133,	100,102,102,113,	43, 48,	40
174	70.7	86, 94,105,125,	55, 88, 96,105,	64, 67,	20
176	76.3	52, 74, 92,105,	82, 87, 89, 95,	78, 83,	10

Day 177. Perfuser drained and refilled with 250 ml. of 80 ppm. 2,4-DCP solution. Solution sampled before adding to the perfuser and after 1 hr. perfusion.

Day.	177ba,	177alhr.,	178b ,
Colourimeter Reading.	42.5,	31.1 ,	12.1,
2,4-DCP conc. in perfusate.(ppm.).	85 ,	62 ,	24 ,

Day 178. Perfuser drained and refilled with 250 ml. of 120 ppm. 2,4-DCP solution. Solution sampled before adding to the perfuser and after 1 hr. perfusion.

Day.	178ba,	178alhr,	179b,
Col.Read.	59 ,	45 ,	25 ,
PPM.2,4-DCP.	118 ,	90 ,	50 ,

Day 179. Perfuser drained and refilled with 100 ppm. 2,4-DCP solution (250 ml.). Solution sampled before adding to the perfuser and after 1 hr. perfusion.

Day.	179ba,	179alhr.,	180,	181,	182,	183,	184,	185,
Col.Read.	49 ,	43 ,	28,	31,	25,	26.5,	23.3,	24,
PPM.2,4-DCP.	98 ,	86 ,	56,	62,	50,	53 ,	46.5,	48,

Table 31. Direct perfusion of 2,4-dichlorophenoxyacetic acid.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm.as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Indicated 2,4-D concentration in the perfusate (ppm.).

Perfusion started on 31/1/52 with 750 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1952,) and 4 litres of 100 ppm. 2,4-D solution. First sample taken after 1 hr. perfusion.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
0	67.0	72, 93, 94, 97,	15, 16, 19, 24,	6,7.5,	120
4	65.0	65, 72, 74, 82,	19, 22, 22, 28,	3, 12,	100
7	61.4	64, 65, 75, 83,	16, 17, 19, 20,	1.5,6.5,	120
10	61.8	57, 57, 63, 63,	16, 18, 19, 21,	3.5,3.5,	120
13	87.3	76, 77, 94, 95,	20, 23, 23, 29,	3.5,4.5,	95
16	57.0	106,107,128,139,	74, 76, 81, 84,	69, 83,	10
19b	70.1	93, 93, 97,120,	106,107,110,137,	109,117,	1

Perfuser drained and refilled with 3,500 ml. of approx. 100 ppm. 2,4-D solution giving a total perfusate volume of 4,000 ml. Perfuser re-started, sampled after 1 hr. Day 19.

19a	75.2	51, 68, 89, 95,	17, 18, 20, 28,	5.5,6.5,	105
21	69.5	66, 72, 76,102,	23, 24, 26, 26,	8.5,8.5,	80
23	64.1	97,100,109,114,	86, 92,102,122,	42, 70,	3
25	63.7	110,116,116,122,	61, 64, 83,100,	38, 38,	5

Day 26, perfuser drained and refilled as on day 19.

26a	63.7	53, 57, 58, 72,	19, 24, 27, 35,	1.5, 3,	90
28	67.4	80, 88, 89,122,	57, 60, 60, 63,	28, 37,	6
30b	61.2	75, 82, 90,111,	105,113,119,134,	121,136,	1

Day 30, perfuser drained and refilled as on day 19.

30a	53.4	71, 71, 88, 88,	22, 22, 24, 26,	5.5,5.5,	100
32	69.3	93, 94,114,124,	93,100,107,108,	56, 75,	2
33b	58.0	124,138,147,156,	142,143,156,167,	64, 71	2

Day 33, perfuser drained and refilled as on day 19.

33a	71.1	55, 68, 68, 80,	11, 12, 13, 17,	3, 4,	100
35	71.1	100,117,125,130,	68, 68, 72, 79,	48, 49,	3

Day 37, perfuser drained and refilled with sufficient water and 2,4-D to make a total perfusate of approximately 150 ppm. 2,4-D.

37a	84.3	69, 79, 87, 88,	14, 14, 17, 19,	1,2.5,	160
39	69.9	86, 92, 96,130,	79, 83, 84, 84,	15, 20	10
41	70.0	101,101,107,116,	103,106,119,120,	113,113,	1

Table 31. continued.

Day 43, perfuser drained and refilled with sufficient water and 2,4-D to make a total perfusate of approximately 4,000 ml. of 200 ppm. 2,4-D. Perfuser re-started, sampled after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
43a	65.3	48,	51,	58,	72,	14,	14,	17,	18,	3,	6,	190
45	64.4	67,	79,	87,	98,	33,	33,	34,	37,	4.5,	4.5,	60
47	72.5	105,	105,	109,	116,	97,	99,	101,	105,	94,	95,	1

Day 52, perfuser drained and refilled with sufficient water and 2,4-D to make a total perfusate of approximately 4,000 ml. of 250 ppm. 2,4-D. Perfuser re-started, sampled after 1 hr.

52a	60.3	52,	52,	67,	77,	8.5,	8.5,	8.5,	10,	1.5,	3.5,	270
54	69.4	60,	67,	81,	106,	13,	13,	14,	16,	1.5,	1.5,	210
56	74.2	81,	100,	100,	108,	65,	68,	73,	95,	41,	93,	3
58	69.6	82,	92,	92,	98,	76,	79,	86,	99,	51,	61,	3

Table 32. Direct perfusion of 2,4-D, followed by POAA.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Longest roots at nominal concentration of 100 ppm. as % m.c.
G. Indicated 2,4-D concentration in the perfusate (ppm.).
H. Indicated POAA concentration in the perfusate (ppm.).

Perfusion started on 20/7/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 100 ppm. 2,4-D solution. Sampled before adding to the perfuser (common stock with perfuser in Table 36.).

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	98.4	88,	90,	102,	106,	23,	23,	24,	26,	3,	4,	95
6	99.8	88,	89,	92,	95,	30,	33,	34,	34,	5,	5,	60
12	98.4	96,	96,	97,	100,	33,	35,	35,	42,	5,	6,	55
18	93.8	96,	97,	101,	106,	102,	104,	109,	118,	93,	96,	<1

Day 21. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

21a	100.3	95,	95,	96,	99,	28,	29,	32,	33,	4,	6,	70
23	100.3	92,	92,	97,	111,	40,	40,	42,	46,	9,	11,	40
25b	99.8	98,	99,	103,	106,	92,	93,	94,	103,	86,	90,	<1

Day 25. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

25a	102.7	88,	90,	92,	94,	24,	28,	28,	33,	4,	5,	75
27	100.0	98,	100,	103,	113,	93,	94,	94,	97,	96,	100,	<1

Day 29. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

29a	96.3	82,	85,	87,	90,	26,	30,	33,	33,	3,	5,	85
31	96.3	95,	96,	98,	103,	95,	95,	98,	98,	103,	112,	<1

Day 33. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

33a	95.2	87,	91,	95,	97,	22,	24,	25,	26,	4,	4,	80
35	95.2	103,	103,	108,	109,	106,	107,	108,	116,	106,	111,	<1

Day 37. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

37a	100.8	87,	89,	89,	92,	27,	32,	33,	33,	5,	6,	65
39	96.1	95,	101,	105,	105,	101,	101,	108,	110,	97,	99,	<1

Day 40. Perfuser drained and refilled with 250 ml. of 100 ppm. POAA solution. Perfuser re-started, sampled after 1 hr.

A.	B.	F.	F.	H.	A.	B.	F.	F.	H.
40a	99.1	22,	24,	95,	44	97.3	21,	23,	100,

Table 33. Direct perfusion of 2,4-D, followed by 2-CPA.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length, (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Longest roots at nominal concentration of 10 ppm. as % m.c.
G. Longest roots at nominal concentration of 100 ppm. as % m.c.
H. Indicated 2,4-D concentration in the perfusate (ppm.).
I. Indicated 2-CPA concentration in the perfusate (ppm.).

Perfusion started on 21/12/51 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried June 1951,) and 250 ml. of 100 ppm. 2,4-D solution. Sampled after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	H.
0	74.3	54,	58,	65,	65,	19,	20,	20,	24,	1.5,	4,	110
10	74.3	57,	63,	66,	85,	15,	15,	22,	22,	2.5,	4,	105
14	70.8	45,	52,	68,	68,	21,	24,	25,	27,	4,	8.5,	90
16	65.5	61,	75,	84,	95,	21,	21,	26,	43,	4.5,	11,	110
18	72.0	61,	63,	65,	81,	17,	22,	28,	32,	7,	8.5,	100
20	71.6	74,	76,	98,	98,	29,	35,	41,	49,	5.5,	7,	50
22	60.8	69,	76,	86,	86,	71,	72,	79,	102,	71,	79,	1
24b	59.7	84,	87,	89,	125,	84,	91,	95,	120,	120,	138,	<1

Day 24. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

24a	59.7	68,	68,	80,	108,	25,	25,	36,	36,	5.5,	6.5,	80
26	59.1	102,	115,	122,	144,	59,	61,	78,	81,	12,	13,	20
28	66.3	68,	71,	78,	98,	68,	77,	77,	101,	71,	84,	1
30b	51.2	88,	109,	125,	148,	101,	119,	125,	157,	80,	96,	<1

Day 30. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

30a	51.2	61,	64,	74,	86,	22,	22,	25,	25,	4,	6,	100
32	43.2	79,	91,	107,	112,	79,	81,	86,	88,	32,	32,	105
34b	49.9	82,	106,	116,	128,	100,	100,	104,	106,	72,	100,	<1

Day 34. Perfuser drained and refilled with 250 ml. of 100 ppm. 2-CPA solution. Perfuser restarted, sampled after 1 hr.

A.	B.	E.	E.	E.	E.	F.	F.	F.	F.	G.	G.	I.
34a	49.9	48,	52,	62,	64,	14,	18,	22,	24,	2,	4,	80
37	77.7	41,	45,	49,	62,	16,	17,	22,	25,	2.5,	4,	65
40	86.6	28,	31,	44,	46,	14,	16,	18,	20,	2.5,	3.5,	100
43	46.6	41,	49,	58,	62,	15,	18,	20,	22,	2,	4.5,	75
45	65.0	52,	54,	55,	72,	18,	20,	20,	20,	3,	3,	70
48	61.4	41,	44,	46,	55,	18,	19,	21,	21,	5,	11,	80

Table 33. continued.

A.	B.	E.	E.	E.	E.	F.	F.	F.	F.	G.	G.	I.
51	61.8	49,	52,	60,	79,	18,	21,	24,	26,	3,	9.5,	60
54	87.3	45,	45,	48,	58,	14,	15,	15,	19,	2.5,	3.5,	100
57	57.0	53,	63,	67,	67,	16,	19,	20,	33,	3.5,	3.5,	65
60	70.1	61,	74,	76,	86,	17,	18,	20,	21,	3,	4.5	75
63	56.9	42,	46,	58,	69,	21,	23,	26,	28,	7,	11	55
66	66.3	45,	55,	56,	56,	13,	19,	21,	31,	4.5,	6.5,	60
69	67.4	55,	57,	58,	71,	19,	22,	24,	25,	3,	3,	55
72	69.3	55,	55,	57,	59,	14,	16,	17,	20,	1.5,	3,	90
75	58.0	64,	64,	67,	71,	17,	17,	19,	24,	5,	5,	60

Table 33a. Direct perfusion of 2,4-D, followed by 2-CPA.

Key to columns in table:
As in Table 33, above.
Details of perfusion set-up, as in Table 33, above.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	H.
0	74.3	71,	83,	84,	90,	20,	23,	24,	26,	2.5,	4,	90
9	52.3	99,	99,	101,	130,	31,	33,	35,	40,	4,	5.5,	60
12,	81.3	47,	58,	60,	65,	65,	66,	66,	83,	12,	22,	20
15b	77.7	82,	84,	94,	125,	72,	81,	84,	84,	90,	93,	<1

Day 15. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser restarted, sampled after 1 hr.

15a	77.7	66,	73,	80,	94,	19,	22,	22,	23,	2.5,	9,	100
17	57.5	112,	122,	122,	124,	45,	49,	52,	68,	14,	16,	25
19	71.6	78,	90,	91,	92,	76,	77,	88,	105,	81,	101,	<1
21	59.0	82,	107,	110,	156,	90,	105,	116,	116,	87,	110,	<1

Day 23. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

23a	52.8	89,	89,	97,	114,	23,	23,	27,	36,	5.5,	9.5,	80
25	66.3	80,	87,	103,	104,	26,	33,	38,	44,	10,	12,	60
27	47.2	68,	72,	87,	100,	51,	59,	64,	64,	19,	19,	20
29	59.0	68,	78,	84,	120,	61,	63,	71,	85,	112,	114,	<1

Day 30. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

30a	51.2	74,	74,	76,	104,	15,	19,	20,	22,	4,	6,	120
32	43.2	88,	95,	112,	118,	93,	95,	95,	97,	23,	30,	6
34b	49.9	96,	104,	106,	124,	84,	86,	90,	122,	36,	86,	<1

Day 34. Perfuser drained and refilled with 250 ml. of 100 ppm. 2-CPA solution. Perfuser re-started, sampled after 1 hr.

Table 33a. continued.

A.	B.	E.	E.	E.	E.	F.	F.	F.	F.	G.	G.	I.
34a	49.9	40,	48,	50,	56,	14,	18,	18,	26,	2,	4,	75
37	77.7	39,	39,	39,	40,	18,	21,	22,	23,	2.5,	5,	80
40	86.6	38,	40,	43,	47,	11,	13,	15,	16,	2.5,	3.5,	125
43	46.6	41,	49,	52,	62,	12,	14,	15,	17,	4.5,	8.5,	70
45	65.0	48,	48,	49,	51,	18,	19,	20,	23,	3,	7.5,	80
48	61.4	59,	60,	63,	65,	24,	24,	26,	28,	11,	16,	45
51	61.8	39,	45,	52,	55,	21,	26,	31,	42,	5,	5,	45
54	87.3	60,	60,	63,	76,	17,	18,	19,	20,	3.5,	4.5,	70
57	57.0	56,	56,	60,	70,	19,	21,	23,	24,	3.5,	5.5,	55
60	70.1	54,	56,	56,	61,	17,	18,	19,	21,	3,	5.5,	70
63	56.9	56,	58,	58,	60,	12,	14,	14,	14,	3.5,	7,	100
66	62.3	56,	61,	65,	85,	16,	19,	20,	21,	3,	3,	70
69	67.4	48,	64,	64,	66,	19,	22,	22,	34,	4.5,	10,	60
72	69.3	49,	59,	68,	69,	17,	18,	18,	26,	3,	4.5,	60
75	58.0	50,	55,	66,	66,	15,	16,	17,	19,	3.5,	3.5,	75

Table 33b. Transferred adaptation to 2,4-D, followed by 2-CPA.

Key to columns in table:
As in Table 33, above.

Perfusion started on 25/3/52 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1952,) and 250 ml. of 100 ppm. 2,4-D solution made up in the combined "active" perfusates drained from other 2,4-D perfusers (Tables 35a, 35b, Day 32,). First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	H.
0	56.0	77,	91,	107,	139,	19,	20,	21,	21,	2,	3.5,	115
5	68.3	63,	63,	73,	79,	13,	14,	18,	25,	3,	4.5,	120
8	68.5	70,	80,	110,	114,	25,	25,	25,	29,	3,	6,	80
11	59.8	83,	88,	95,	97,	77,	95,	108,	123,	80,	82,	1

Day 15. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

15a	79.0	80,	82,	89,	94,	18,	20,	23,	24,	2.5,	4,	100
33	69.8	73,	94,	109,	123,	89,	90,	102,	104,	73,	74	1

Day 35. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

35a	66.8	90,	99,	107,	129,	19,	21,	21,	24,	3,	4.5,	110
37	69.8	79,	83,	87,	106,	22,	23,	29,	39,	4.5,	4.5,	75
39	79.1	100,	101,	106,	118,	37,	42,	61,	88,	66,	85,	1
41	56.0	118,	122,	127,	131,	91,	102,	107,	118,	72,	90,	<1

Table 33b. continued.

Day 43. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	H.
43a	64.8	82, 90, 96,105,	25, 28, 31, 32,	4.5, 6,	70
45	58.3	76, 95,101,115,	74, 79, 96,120,	24, 88,	6
47	70.5	60, 69, 70, 90,	76, 79, 83,100,	61,109,	<1

Day 48. Perfuser drained and refilled with 250 ml. of 100 ppm. 2-CPA solution. Perfuser re-started, sampled after 1 hr.

A.	B.	D. D. D. D.	E. E. E. E.	F. F.	I.
48	56.6	106,115,122,138,	60, 66, 87, 94,	17, 27,	55
53	68.2	71, 76, 85,130,	46, 47, 59, 63,	16, 18,	90
58	66.6	69, 86, 98,110,	33, 36, 45, 48,	18, 24,	80
63	61.6	70, 75, 82, 96,	46, 49, 73, 91,	19, 23,	60
68	42.2	78, 83, 85, 97,	43, 45, 55, 57,	21, 26,	90
73	44.0	66, 71, 75,114,	64, 66, 68, 77,	25, 27,	40
78	41.3	82, 92,102,119,	61, 92,102,104,	12, 39,	50
83	51.4	86,107,130,148,	49, 56, 68, 85,	25, 29,	40
88	52.5	59, 63, 67, 80,	53, 61, 61, 63,	15, 23,	70
93	59.4	77, 86,113,113,	59, 64, 72, 82,	17, 19,	55
103	52.5	57, 88, 95,118,	50, 63, 76, 89,	32, 34,	25
113	52.1	61, 61, 69, 88,	61, 63, 65, 73,	25, 29,	40
118	46.8	75, 86, 92, 98,	51, 51, 68, 75,	24, 39,	75

Table 33c. Transferred adaptation to 2,4-D, followed by 2,CPA.

Key to columns in table:
As in Table 33, above.
Details of perfusion set-up, as in Table 33b, above.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	H.
0	56.0	75, 84,102,136,	19, 20, 27, 34,	3.5,5.5,	90
5	68.3	41, 51, 66, 76,	14, 17, 19, 20,	3, 3,	120
8	68.5	76, 82, 95,104,	19, 20, 20, 23,	3,4.5,	110
11	59.8	103,117,120,143,	73, 92,112,138,	58, 78,	2

Day 15. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after.1 hr.

15a	79.0	62, 72, 80, 90,	18, 21, 21, 27,	3, 5,	110
33	69.8	100,112,113,114,	69, 70, 73, 80,	83,104,	<1

Day 35. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

Table 33c. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	H.
35a	66.8	55,	65,	84,	89,	21,	23,	23,	24,	6,	9,	100
37	69.8	80,	96,	106,	109,	20,	23,	34,	37,	4.5,	6,	75
39	79.1	77,	81,	89,	101,	81,	89,	92,	93,	48,	56,	3
41	56.0	120,	122,	130,	139,	88,	113,	125,	150,	48,	72,	2

Day 43. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

43a	64.8	62,	63,	65,	66,	25,	25,	28,	29,	3,	4.5,	100
45	58.3	86,	93,	96,	100,	88,	88,	96,	117,	86,	88,	<1
47	70.5	80,	83,	93,	99,	77,	81,	89,	115,	69,	71,	1

Day 48. Perfuser drained and refilled with 250 ml. of 100 ppm. 2-CPA solution. Perfuser re-started, sampled after 1 hr.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	I.
48a	56.6	66,	67,	102,	136,	51,	57,	66,	78,	10,	19,	100
53	68.2	66,	68,	73,	128,	29,	34,	35,	47,	17,	25,	110
58	66.6	80,	81,	89,	104,	35,	39,	51,	65,	13,	24,	90
63	61.6	91,	91,	114,	120,	52,	57,	57,	72,	11,	29,	65
68	42.2	128,	130,	150,	159,	40,	50,	55,	81,	26,	31,	70
73	44.0	70,	73,	80,	100,	43,	45,	48,	59,	16,	34,	100
78	41.3	109,	143,	146,	148,	68,	78,	78,	121,	27,	27,	35
83	51.4	80,	91,	101,	119,	56,	58,	64,	76,	8,	13,	55
88	52.5	80,	107,	122,	133,	53,	55,	55,	57,	19,	21,	65
93	59.4	84,	94,	103,	113,	62,	62,	71,	79,	13,	22,	90
103	52.5	59,	63,	80,	84,	40,	51,	63,	65,	13,	19,	100
113	52.1	86,	102,	111,	111,	60,	60,	67,	85,	27,	33,	40
118	46.8	100,	107,	111,	122,	75,	86,	88,	105,	24,	26,	45

Table 34. Direct perfusion of 2,4-D / 2-CPA mixture.

Key to columns in table:

A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. (with respect to the 2,4-D component) as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. (with respect to the 2,4-D component) as percentage mean control.
E. Longest roots at nominal concentration of 1.0 ppm. (with respect to the 2,4-D component) as percentage mean control.
F. Total indicated activity of the perfusate as ppm. 2,4-D.

Perfusion started on 8/2/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of solution (common with Table 34a,) containing 100 ppm. each of 2,4-D and 2-CPA. Solution sampled before adding to the perfusers.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	84.2	46,	59,	63,	63,	15,	16,	17,	18,	3.5,	3.5,	170
5	76.3	43,	47,	56,	59,	17,	18,	18,	19,	4,	5,	150
10	82.6	56,	57,	65,	75,	15,	16,	18,	21,	2.5,	3.5,	170
15	84.1	35,	36,	45,	76,	14,	15,	16,	17,	2.5,	3.5,	180
18	88.2	69,	78,	81,	88,	13,	14,	16,	17,	3.5,	4.5,	165
21	82.0	93,	105,	107,	118,	49,	51,	51,	52,	28,	29,	30
24	86.1	84,	85,	88,	88,	60,	63,	67,	77,	40,	40,	5
27	67.8	100,	112,	116,	116,	71,	74,	85,	94,	41,	41,	5
30b	92.9	71,	72,	81,	84,	74,	77,	91,	95,	37,	41,	5

Day 30. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 500 ppm. 2-CPA. Perfuser re-started, sampled after 1 hr.

30a	92.9	40,	47,	48,	52,	14,	15,	16,	22,	2,	3,	175
36	71.0	72,	87,	89,	97,	24,	24,	27,	34,	7,	10,	80
39	77.0	82,	84,	91,	97,	29,	30,	40,	40,	10,	12,	50
42	73.3	62,	54,	68,	71,	27,	31,	33,	33,	12,	17,	15
45	89.4	67,	69,	70,	97,	52,	55,	56,	61,	13,	14,	20
48b	84.6	73,	90,	97,	106,	41,	47,	53,	53,	12,	16,	20

Day 48. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 1,000 ppm. 2,CPA. Perfuser re-started, sampled after 1 hr.

48a	83.1	46,	47,	48,	58,	12,	12,	14,	16,	2.5,	3.5,	235
51	83.1	40,	44,	46,	48,	18,	18,	30,	31,	3.5,	6,	140
54	72.1	36,	39,	43,	53,	18,	18,	21,	21,	4,	5.5,	125
57	78.2	43,	47,	48,	59,	15,	16,	17,	19,	5,	5,	145
60	83.4	61,	63,	69,	75,	17,	17,	19,	22,	5,	6,	135
63	72.1	66,	66,	66,	83,	21,	22,	25,	26,	4,	4,	90
66	80.7	44,	55,	62,	68,	24,	24,	25,	35,	6,	7.5,	90
69	74,9	51,	51,	53,	55,	27,	28,	32,	37,	5.5,	8,	70
72	82.1	73,	73,	76,	90,	19,	23,	29,	34,	5,	7.5,	80
78	69.9	84,	86,	89,	101,	33,	33,	37,	44,	8.5,	12,	50

Day 78. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

Table 34. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
78a	69.9	64,	68,	70,	74,	20,	21,	21,	26,	4.5,	4.5,	110
84	75.5	93,	94,	97,	102,	86,	93,	95,	110,	57,	59,	2.5

Table 34a. Direct perfusion of 2,4-D / 2-CPA mixture.

Key to columns in table: as for Table 34, above.
Details of perfusion set-up, as for Table 34, above.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	84.2	46,	59,	63,	63,	15,	16,	17,	18,	3.5,	3.5,	170
5	76.3	55,	59,	65,	65,	14,	17,	17,	19,	4,	4,	160
10	82.6	29,	29,	30,	33,	12,	13,	14,	15,	5,	6,	185
15	84.1	46,	50,	59,	65,	14,	15,	16,	17,	2.5,	3.5,	185
18	88.2	59,	61,	67,	69,	20,	22,	22,	23,	3.5,	5.5,	105
21	82.0	54,	55,	58,	75,	40,	49,	63,	63,	21,	22,	15
24	86.1	72,	85,	85,	93,	79,	89,	93,	95,	35,	53,	5
27	67.8	83,	84,	88,	102,	77,	81,	87,	94,	43,	53,	3
30b	92.9	79,	81,	85,	85,	68,	68,	82,	83,	53,	58,	2.5

Day 30. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 500 ppm. 2-CPA. Perfuser re-started, sampled after 1 hr.

30a	92.9	46,	46,	61,	62,	13,	15,	15,	16,	3,	4.5,	185
36	71.0	60,	73,	76,	87,	24,	24,	25,	31,	4,	7,	90
39	77.0	75,	79,	80,	89,	22,	23,	30,	34,	8,	9,	80
42	73.3	50,	54,	56,	87,	27,	29,	29,	37,	8,	8,	70
45	89.4	72,	77,	78,	98,	29,	31,	38,	45,	13,	15,	55
48b	84.6	73,	74,	74,	79,	45,	46,	50,	51,	13,	13,	35

Day 48. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 1,000 ppm. 2-CPA. Perfuser re-started, sampled after 1 hr.

48a	83.1	35,	35,	41,	48,	13,	14,	15,	20,	2.5,	2.5,	200
51	83.1	65,	65,	66,	66,	13,	14,	15,	19,	3.5,	3.5,	180
54	72.1	72,	76,	83,	101,	21,	22,	25,	33,	7,	9.5,	85
57	78.2	51,	54,	56,	70,	19,	20,	22,	23,	5,	6.5,	100
60	83.4	69,	73,	74,	75,	20,	20,	22,	23,	5,	6,	110
63	72.1	65,	65,	69,	72,	22,	25,	25,	26,	8.5,	8.5,	85
66	80.7	58,	63,	63,	69,	32,	38,	42,	43,	8.5,	10,	50
69	74.9	63,	73,	85,	87,	28,	29,	29,	35,	9.5,	12,	70
72	82.1	78,	79,	87,	94,	24,	27,	27,	28,	8.5,	12,	80
78b	69.9	86,	94,	100,	100,	39,	43,	46,	53,	13,	17,	40

Day 78. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

78a	69.9	80,	84,	86,	110,	21,	29,	29,	41,	3,	4.5,	85
84	75.5	103,	103,	104,	113,	98,	103,	103,	104,	66,	68,	2

Table 35. Transferred adaptation to 2,4-D,followed by 4-CPA.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Indicated 2,4-D concentration in the perfusate (ppm.).
G. Indicated 4-CPA concentration in the perfusate (ppm.).
H. Colourimeter reading (E.E.L.) (scale divisions).
I. Indicated "phenols" concentration in the perfusate, as ppm. 4-CP.

Perfusion started on 20/1/52 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried June 1951,) and 250 ml. of 100 ppm. 2,4-D solution prepared from the"active" perfusate drained from a 2,4-D enriched perfuser. Sampled after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	51.2	66,	68,	76,	84,	27,	27,	33,	35,	6,	6,	90
5	58.1	53,	55,	60,	102,	22,	28,	28,	29,	1.5,	3.5,	100
8	74.6	68,	68,	75,	80,	24,	24,	25,	29,	4,	6.5,	90
11	67.0	52,	57,	96,	99,	72,	84,	84,	90,	94,	124,	<1
14b	57.1	107,	110,	110,	114,	112,	119,	131,	133,	116,	118,	<1

Day 14. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

14a	57.1	95,	98,	103,	105,	25,	26,	28,	30,	3.5,	14,	75
17	72.6	62,	72,	84,	106,	75,	84,	84,	112,	15,	32,	10
18	61.4	88,	101,	101,	127,	85,	96,	102,	111,	47,	52,	3
20b	68.4	67,	92,	101,	108,	60,	94,	104,	111,	78,	99,	<1

Day 20. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

20a	68.4	48,	50,	53,	72,	19,	20,	22,	23,	4.5,	6,	105
23	60.9	86,	89,	109,	131,	95,	100,	102,	107,	23,	63,	5
25	75.2	67,	83,	88,	103,	73,	75,	79,	85,	55,	107,	<1
26b	57.0	86,	93,	107,	112,	128,	134,	142,	142,	102,	114,	<1

Day 26. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

26a	66.4	49,	54,	55,	63,	13,	18,	18,	19,	4.5,	6,	125
28	86.7	69,	89,	100,	107,	69,	83,	83,	95,	16,	18,	15
30	70.1	106,	107,	110,	113,	101,	107,	117,	129,	97,	99,	<1

Day 31. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr.

31a.	75.2	81,	88,	91,	96,	28,	31,	37,	40,	4,	6.5,	65

Table 35. continued.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
34	64.1	81, 92,100,118,	42, 45, 48, 53,	6, 6,	30
36	62.3	93,103,106,112,	121,124,126,145,	85, 89,	<1
38b	67.4	91, 95, 97,102,	89, 98,120,137,	64, 73,	<1

Day 38. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr,

38a	67.4	71, 74, 86, 89,	21, 24, 28, 39,	4.5, 9,	90
40	55.1	107,111,125,134,	38, 44, 53, 54,	9, 15,	30
42	69.3	98,101,103,133,	87, 91, 91,103,	72,101,	<1
44	61.2	88,102,113,114,	49, 79, 95,106,	79, 79,	1

Day 46. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 4-CPA and 10 ppm. sodium azide. Perfuser re-started, sampled after 1 hr.

46a	71.1	76, 83, 87,118,	20, 23, 27, 44,	5.5, 14,	85
48	67.5	93, 96,102,119,	27, 28, 33, 58,	6.0,7.5,	55
50	85.9	113,113,119,119,	102,107,112,113,	102,111,	<1

Day 54. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. of 4-CPA and 100 ppm. sodium fluoride. Perfuser re-started, sampled after 1 hr.

54a	77.5	70, 79, 84, 88,	21, 22, 25, 49,	4, 4,	90
56	64.4	79, 92, 93,111,	86, 90,112,134,	106,111,	<1

Day 59. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 4-CPA and 1,000 ppm. sodium fluoride. Perfuser re-started, sampled after 1 hr.

59a	72.5	62, 66, 80, 84,	19, 21, 23, 37,	7, 12,	95
61	62.8	64, 65, 94, 99,	105,109,112,126,	91, 99,	<1

Day 64. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 4-CPA and 5,000 ppm. sodium fluoride. Perfuser re-started, sampled after 3 hr. In this time the perfusate had become dark brown in colour.

64a	69.6	87, 99,102,106,	22, 22, 27, 34,	6, 7,	105
66	69.4	53, 84,104,108,	35, 35, 36, 68,	4.5, 13,	55
68	74.2	86, 93,103,135,	45, 58, 62, 65,	9.5, 11,	30
70	69.6	53, 56, 63, 92,	54, 59, 93,114,	19, 40,	10
72	60.9	77, 86,102,109,	66, 91, 96, 99,	92, 97,	1

Day 74. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 4-CPA and 5,000 ppm. sodium fluoride. Perfuser re-started, sampled after 1 hr.

74a	59.8	55, 60, 65, 75,	28, 30, 32, 38,	5, 5,	100
76	59.8	67, 77, 99,108,	18, 22, 27, 32,	6.5, 10,	80

Table 35. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
78	72.4	62,	76,	79,	107,	23,	26,	28,	28,	4,	5.5,	90
80	72.4	69,	95,	98,	127,	35,	35,	36,	37,	4,	7,	55
99	67.0	87,	94,	100,	106,	48,	51,	54,	57,	13,	15,	25
105	67.0	126,	127,	136,	141,	60,	67,	69,	69,	15,	25,	15
111	56.6	90,	94,	94,	127,	65,	73,	90,	108,	12,	14,	10

"Phenols" as ppm. 4-CP.

A.	H.	I.
54a	0	0
55	1.4	1.9
56	3.4	4.5
57	0.4	0.5
58	1.4	1.9
59a	0.4	0.5
60	3.8	5.1
61	6.0	8.0
62	8.1	10.8
63	7.2	9.6
64b	4.5	6.0
64b.add 0	0	0
64a.3hr 45	60	
65	91	121
66	109	145
67	110	146
68	115	153
69	112	149
70	115	153
71	117	156
72	120	160
73	122	163

A.	H.	I.
74b.add 0	0	
74a.1hr 14.3	19	
75	23.1	30.5
76	23.4	31.0
77	30	40
78	29.3	39.0
79	31.1	41.5
80	39	52
98	70	93
99	74	98.5
100	77	102
101	73	97
102	76	101
103	78	104
104	88	117
105	90	120
106	89.5	119
107	84	112

The perfusate on Day 115 was steam-distilled and the first 100 ml. of distillate collected and tested for phenols. The equivalent of about 1.5 ppm., as 4-CP, was found when related back to the perfusate. This figure suggests that the high apparent figures obtained on directly testing the perfusate were false and not due to the presence of phenols. In any case, the indicated phenol could not be 4-CP for even a quantitative conversion would only give 75 ppm. 4-CP from 100 ppm. 4-CPA.

Table 35a. Transferred adaptation to 2,4-D, followed by 4-CPA.

Key to columns in table: as in Table 35.

Perfusion started on 21/2/52 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1952,) and 250 ml. of 100 ppm. 2,4-D solution prepared from the "active" perfusate drained from two 2,4-D enriched perfusers (Tables 35, and 35c,) on 20/2/52. Solution common with that of Table 35b. Sampled after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	56.9	60,	65,	79,	99,	25,	30,	33,	35,	7,	11,	65
3	63.5	55,	58,	71,	101,	19,	22,	24,	24,	3,	6.5,	90
6	58.1	84,	102,	110,	124,	31,	34,	36,	46,	5,	7,	55,
8	55.1	104,	107,	116,	129,	42,	51,	69,	76,	12,	15,	25
11	69.3	65,	90,	94,	123,	87,	101,	101,	101,	75,	78,	1
13	58.0	119,	121,	123,	128,	92,	95,	109,	111,	83,	85,	<1

Day 14. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

14a	71.1	76,	80,	80,	84,	18,	19,	21,	21,	3,	4,	105
16	69.4	82,	85,	115,	119,	24,	29,	30,	36,	4.5,	6,	70
18	85.9	90,	99,	120,	138,	42,	44,	45,	60,	7,	15,	35
19	71.9	110,	112,	114,	128,	102,	110,	111,	130,	18,	22,	10
21	70.0	104,	111,	114,	127,	99,	100,	103,	111,	106,	109,	<1

Day 23. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

23a	65.3	69,	83,	84,	97,	20,	20,	22,	28,	3,	4.5,	110
25	64.4	65,	80,	93,	104,	25,	25,	30,	34,	4.5,	9.5,	80
27	72.5	87,	87,	90,	104,	64,	72,	73,	76,	46,	80,	2.5

Day 32. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
3 2a	60.3	78,	78,	83,	90,	22,	25,	27,	32,	6.5,	8.5,	95
34	69.4	76,	81,	89,	97,	29,	29,	32,	34,	4.5,	6,	80
36	74.2	42,	46,	72,	72,	32,	32,	34,	35,	2.5,	4,	65
38	69.6	55,	59,	90,	99,	50,	50,	55,	60,	10,	15,	25
40	60.9	61,	66,	76,	105,	100,	102,	104,	116,	97,	122,	<1

Day 42. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr.

42a	66.6	93,	101,	110,	111,	26,	32,	36,	39,	1.5,	1.5,	60
44	59.8	100,	100,	107,	108,	55,	63,	95,	113,	25,	30,	10
46	58.8	118,	126,	138,	141,	87,	89,	100,	106,	118,	123,	<1

Table 35b. Transferred adaptation to 2,4-D, followed by 4-CPA.

Key to columns in table: as in Table 35.
Details of perfusion set-up: as in Table 35a.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
0	56.9	69, 71, 88, 92,	23, 25, 28, 30,	3.5,3.5,	85
3	63.5	76, 79, 87, 87,	19, 19, 20, 21,	4.5, 8,	80
6	58.1	96,103,105,138,	29, 33, 33, 47,	5, 5,	60
8	55.1	104,109,111,140,	123,125,134,138,	105,124,	<1

Day 10. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

10a	69.3	59, 79, 87, 95,	20, 22, 22, 39,	4.5,8.5,	105
12	61.2	59, 64, 67,107,	28, 29, 31, 43,	3.5,6.5,	70
14	71.1	106,107,115,118,	96,107,107,124	121,130,	<1
16	69.4	111,112,122,128,	68,105,108,109,	56, 83,	<1

Day 17. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

17a	84.3	72, 88, 97,119,	15, 16, 17, 19,	1,2.5,	120
19	71.9	84, 85,106,106,	22, 22, 24, 25,	5.5,8.5,	90
21	70.0	90, 91,100,110,	84,103,104,104,	93,107,	1

Day 23. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

23a	65.3	58, 61, 61, 91,	21, 25, 28, 31,	3,4.5,	90
25	64.4	58, 62, 73, 84,	31, 33, 34, 40,	3,4.5,	65
27	72.5	102,105,105,106,	94, 95,109,123,	48, 76,	2

Day 32. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	G.
32a	60.3	60, 65, 72, 95,	30, 35, 35, 50,	5,6.5,	90
34	69.4	57, 83,101,124,	23, 24, 37, 39,	6, 10,	75
36	74.2	72, 74, 76,104,	32, 35, 36, 38,	5.5, 7,	55
38	69.6	80, 86, 90,109,	53, 60, 92, 99,	20, 26,	10
40	60.9	94, 96,100,104,	96, 96,107,116,	82, 84,	<1

Day 42. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr.

42a	66.6	90,101,111,119,	29, 29, 33, 35,	4.5,4.5,	80
44	59.8	73, 87,100,137,	65, 70, 72, 72,	15, 18,	15
46	58.8	104,109,126,140,	90, 94,109,116,	104,107,	1

Table 35c. Transferred adaptation to 2,4-D, followed by 4-CPA.

Key to columns in table: as in Table 35.

Details of perfusion set-up: as for Table 35 with a common solution was shared.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	51.2	78,	82,	94,	110,	23,	23,	23,	33,	6,	6,	90
5	58.1	69,	71,	84,	91,	33,	33,	36,	45,	3.5,	5,	60
8	74.6	69,	75,	79,	88,	61,	79,	79,	95,	55,	100,	1
11	67.0	67,	78,	84,	99,	66,	69,	84,	93,	91,	94,	1
Day 14. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.												
14a	57.1	61,	67,	77,	89,	19,	21,	21,	23,	3.5,	5.5,	105
17	72.6	80,	88,	91,	100,	29,	37,	40,	43,	6.5,	8.0,	50
18	61.4	85,	91,	99,	117,	34,	39,	64,	82,	29,	33,	10
21b	61.8	81,	89,	99,	105,	91,	92,	102,	107,	62,	86,	1
Day 21. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.												
21a	61.8	76,	76,	87,	92,	14,	15,	16,	23,	3,	5,	100
24	87.3	85,	90,	91,	100,	85,	91,	94,	96,	46,	61,	25
26b	66.4	70,	79,	109,	112,	87,	103,	109,	111,	72,	84,	1
Day 26. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.												
26a	66.4	51,	51,	52,	64,	21,	21,	23,	28,	4.5,	4.5,	105
28	86.7	89,	89,	95,	110,	83,	94,	105,	114,	18,	21,	10
30	70.1	96,	99,	103,	104,	104,	110,	117,	120,	91,	97,	<1
Day 31. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr.												
A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
31a	75.2	69,	71,	96,	103,	24,	24,	33,	44,	2.5,	2.5,	105
34	64.1	83,	83,	89,	94,	30,	30,	42,	51,	3,	4.5,	80
36	62.3	90,	92,	114,	126,	76,	79,	84,	89,	53,	55,	5
38b	67.4	85,	89,	98,	108,	57,	73,	79,	116,	64,	70,	2.5
Day 38. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr.												
38a	67.4	70,	70,	77,	88,	24,	27,	31,	34,	4.5,	4.5,	90
40	55.1	87,	96,	109,	114,	53,	56,	71,	76,	14,	24,	15
42	69.3	87,	90,	119,	123,	93,	97,	103,	123,	91,	93,	<1
44	61.2	101,	106,	111,	121,	82,	108,	115,	119,	101,	103,	<1
Day 46. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 4-CPA and 1 ppm. NaN_3. Sampled after 1 hr.												
46a	71.1	59,	69,	70,	75,	30,	31,	31,	38,	11,	16,	75
48	67.5	106,	111,	116,	122,	43,	45,	50,	61,	6,	7.5,	45
50	85.9	93,	97,	117,	118,	26,	36,	56,	67,	3.5,	3.5,	50

Table 36. Direct perfusion of 2,4-D, followed by 3,4-D.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Longest roots at nominal concentration of 10 ppm. as % m.c.
G. Indicated 2,4-D concentration in the perfusate (ppm.).
H. Indicated 3,4-D concentration in the perfusate (ppm.).

Perfusion started on 20/7/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 100 ppm. 2,4-D solution. Sampled before adding to the perfuser,(common stock solution with perfuser in Table 32.).

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
0	98.4	88,	90,	102,	106,	23,	23,	24,	26,	3,	4,	95
6	99.8	96,	96,	96,	99,	23,	23,	23,	32,	3,	4,	90
12	98.4	82,	92,	92,	93,	21,	24,	26,	28,	4,	4,	85
18	93.8	95,	101,	102,	105,	97,	100,	101,	102,	97,	110,	<1

Day 21. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

21a	100.3	94,	95,	96,	99,	25,	28,	28,	32,	3,	4,	75
23	100.3	88,	88,	96,	102,	39,	40,	42,	49,	6,	7,	50
25b	99.8	97,	102,	104,	114,	98,	99,	101,	104,	100,	101,	<1

Day 25. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

25a	102.7	90,	94,	96,	96,	22,	22,	25,	28,	5,	6,	70
27	100.0	96,	97,	97,	99,	94,	96,	99,	113,	98,	99,	<1

Day 29. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

29a	96.3	83,	86,	87,	91,	19,	22,	24,	37,	4,	6,	80
31	96.3	95,	102,	103,	104,	101,	102,	112,	113,	95,	100,	<1

Day 33. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser restarted, sampled after 1 hr.

33a	95.2	84,	88,	93,	102,	29,	33,	34,	36,	4,	4,	65
35	95.2	100,	102,	103,	114,	94,	99,	100,	104,	91,	91,	<1

Day 37. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

37a	100.8	84,	84,	87,	95,	29,	31,	31,	33,	4,	6,	70
39	96.1	100,	101,	104,	106,	94,	96,	97,	106,	94,	99,	<1

Day 40. Perfuser drained and refilled with 250 ml. of 100 ppm. 3,4-D solution. Perfuser re-started, sampled after 1 hr.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	H.
40a	97.0	82,	88,	89,	91,	46,	48,	56,	56,	6,	8,	60
44	97.3	92,	94,	100,	103,	40,	43,	48,	56,	4,	5,	60

Table 37. Transferred adaptation to 2,4-D, followed by 2,4,5-T.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Longest roots at nominal concentration of 10 ppm. as % m.c.
G. Indicated 2,4-D concentration in the perfusate (ppm.).
H. Indicated 2,4,5-T concentration in the perfusate (ppm.).

Perfusion started on 10/5/52 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried April 1952,) and 250 ml. of 100 ppm. 2,4-D solution. This solution (common stock with that of Table 37a,) was prepared from the combined "active" perfusates drained from other 2,4-D perfusers (Tables 33b, 33c, on day 43). First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
0	56.6	73,	83,	88,	96,	19,	27,	28,	51,	3.5,	3.5,	80
3	69.0	55,	55,	74,	101,	13,	14,	29,	29,	3,	4.5,	80
6	54.7	58,	58,	60,	92,	20,	20,	24,	35,	5.5,	5.5,	80
9	62.0	97,	99,	102,	118,	78,	79,	100,	134,	73,	107,	<1
12b	66.6	90,	108,	119,	123,	86,	93,	95,	123,	92,	104,	<1
Day 12. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser-re-started, sampled after 1 hr.												
12a	66.6	62,	69,	74,	77,	21,	21,	23,	24,	3,	4.5,	100
15	65.3	81,	83,	84,	103,	86,	97,	109,	114,	51,	74,	2
Day 18. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.												
18a	57.2	32,	42,	45,	68,	19,	23,	26,	47,	3.5,	5,	100
20	57.6	89,	109,	125,	142,	70,	76,	85,	96,	38,	78,	2.5
Day 22. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr,												
22a	42.2	83,	88,	90,	123,	21,	24,	31,	31,	7,	9.5,	70
24	47.7	94,	107,	107,	155,	73,	75,	84,	111,	82,	109,	<1
Day 26. Perfuser drained and refilled with 250 ml. of 10 ppm. 2,4,5-T solution. Perfuser re-started, sampled after 1 hr.												
A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	H.
26a	49.4	57,	59,	73,	93,	22,	22,	26,	28,	4,	6,	6.0
31	49.9	32,	42,	42,	70,	26,	28,	30,	32,	2,	4,	5.0
36	46.5	95,	99,	114,	120,	22,	26,	26,	41,	4.5,	6.5,	5.5

Table 37. continued.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	H.
46	57.7	94,	99,	101,	107,	24,	26,	31,	36,	5,	5,	4.5
56	47.0	94,	102,	104,	113,	19,	21,	23,	26,	6.5,	6.5,	7.0
61	54.0	76,	93,	96,	117,	15,	22,	26,	30,	3.5,	3.5,	6.0

Table 37a. Transferred adaptation to 2,4-D, followed by 2,4,5-T.

Key to columns in table: as in Table 37, above.
Details of perfusion set-up, as in Table 37, above.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
0	56.6	64,	67,	76,	138,	21,	27,	30,	32,	3.5,	7,	90
6	54.5	73,	95,	104,	106,	22,	24,	24,	26,	3.5,	3.5,	90
9	62.0	123,	126,	131,	174,	98,	105,	108,	113,	50,	70,	3
12b	66.6	50,	65,	87,	134,	92,	95,	96,	113,	65,	66,	2

Day 12. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

12a	66.6	47,	60,	60,	63,	18,	21,	27,	35,	4.5,	6,	85
15	65.3	55,	66,	78,	91,	68,	118,	120,	120,	43,	63,	3

Day 18. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

18a	57.2	84,	86,	89,	93,	23,	23,	25,	28,	5,	7,	85
20	57.6	51,	64,	71,	124,	83,	94,	98,	106,	49,	63,	3

Day 22. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

22a	42.2	81,	88,	95,	100,	33,	43,	43,	50,	4.5,	9.5,	55
24	47.7	111,	115,	122,	128,	92,	101,	103,	124,	65,	109,	<1

Day 26. Perfuser drained and refilled with 250 ml. of 10 ppm. 2,4,5-T solution. Perfuser re-started, sampled after 1 hr.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	H.
26a	49.4	85,	89,	99,	120,	17,	20,	20,	28,	2,	4,	8.0
31	49.9	76,	84,	94,	96,	22,	26,	26,	36,	2,	6,	6.0
36	46.5	80,	103,	114,	125,	22,	24,	24,	37,	4.5,	4.5,	6.5
46	57.7	88,	92,	130,	134,	16,	21,	23,	24,	3.5,	5,	7.5
56	47.0	64,	68,	74,	79,	19,	26,	32,	38,	2,	4.5,	7.0
61	54.0	57,	63,	70,	93,	13,	15,	18,	24,	2,	3.5,	9.5

Table 38. Direct perfusion of 2,4-D, followed by MCPA.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Indicated 2,4-D concentration in the perfusate (ppm.).
G. Indicated MCPA concentration in the perfusate (ppm.).

Perfusion started on 25/5/51 with 75 gm. of soil (2 to 4 mm., South Church Lane Allotments, Bishop Auckland, dried January 1951,) and 250 ml. of approximately 1,000 ppm. 2,4-D solution. Solution sampled before adding to the perfuser and after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0b	40.2	80,	80,	90,	110,	32,	32,	42,	55,	5,	5,	700
0a	40.2	75,	80,	82,	85,	32,	35,	57,	57,	5,	13,	650
4	40.2	80,	85,	112,	122,	47,	50,	55,	60,	5,	7.5,	400
14	39.8	68,	74,	83,	88,	43,	43,	60,	86,	5,	7.5,	400
38	46.2	80,	82,	91,	98,	95,	100,	100,	117,	104,	115,	<1

Day 42. Perfuser drained and refilled with 250 ml. of approx. 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

42a	40.6	79,	84,	86,	86,	44,	49,	49,	52,	5,	7.5,	550
44	37.7	90,	93,	93,	101,	53,	53,	58,	79,	5,	11,	400
46	37.2	89,	92,	97,	102,	62,	62,	65,	65,	8,	11,	300
48	35.2	102,	108,	122,	125,	68,	71,	74,	80,	11,	12,	150
50	38.5	73,	75,	78,	91,	78,	81,	88,	96,	36,	44,	70
52	32.8	88,	92,	92,	107,	119,	122,	122,	125,	110,	113,	<10

Day 55. Perfuser drained and refilled with 250 ml. of approx. 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

55a	37.6	75,	77,	80,	106,	59,	61,	61,	64,	8,	8,	400
58	37.4	75,	75,	110,	112,	48,	51,	54,	70,	19,	27,	100
60	37.0	89,	92,	100,	100,	108,	113,	119,	124,	92,	98,	<10

Day 65. 25 ml. of stock 1% 2,4-D solution added to the perfuser without draining to make the perfusate approximately 250 ml. of 1,000 ppm. 2,4-D. Perfuser re-started, sampled after 1 hr.

65a	35.1	91,	100,	100,	108,	60,	63,	68,	68,	5.5,	8.5,	350
67	37.8	80,	90,	93,	95,	48,	50,	53,	56,	5.5,	8,	450
69	42.8	66,	66,	100,	110,	47,	49,	54,	54,	9.5,	7,	300
71	46.6	80,	84,	95,	101,	52,	54,	54,	58,	11,	11,	250
73	44.9	71,	71,	89,	98,	80,	83,	84,	87,	65,	67,	40

Table 38. continued.

Day 76. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
76a	64.2	70, 73, 76,104,	25, 25, 30, 53,	6, 6,	800
79	45.2	104,108,148,155,	55, 58, 60, 67,	13, 18,	250
81	48.9	108,119,123,129,	68, 72, 72, 82,	12, 14,	150
83	67.4	92,100,104,114,	89, 89,100,104,	68, 93,	10
85	67.3	104,104,106,117,	109,115,115,116,	98,112,	<10

Day 87. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started.
Day 88. The perfuser had ceased to function (mechanically) so the perfusate was drained into a sterile flask, shaken with the "active" soil from the column, and the suspension allowed to settle. The opaque supernatant liquid was decanted off through a new 50 gm. soil column (1 to 4 mm., Sussex Lodge soil, dried June 1950,). The perfuser was re-assembled with this column and perfusate. Sampled after 1 hr. perfusion.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	G.
88a	58.7	63, 73, 79, 82,	20, 24, 26, 29,	4, 4,	100
92	77.7	63, 68, 70, 71,	23, 32, 34, 37,	4, 4,	85
94	72.8	81, 85, 85, 92,	25, 30, 34, 45,	4, 8,	75
96	74.8	80, 82, 87, 90,	24, 25, 29, 31,	4, 4,	75
98	70.1	84, 89,100,103,	30, 33, 41, 50,	4, 4,	55
100	56.4	87, 97,102,112,	32, 35, 42, 55,	8, 11,	40
102	60.1	102,108,110,113,	43, 58, 60, 78,	7, 10,	25
104	51.6	110,120,126,144,	47, 47, 47, 55,	14, 16,	20
106	42.3	92,107,109,114,	54, 62, 73, 80,	14, 19,	15
108	45.4	93, 95,104,106,	71, 75, 77, 77,	26, 29,	10
110	55.0	66, 82, 97,120,	62, 64, 64, 71,	26, 26,	10
112	56.0	95, 95,123,146,	89,100,114,116,	43, 55,	3
114	62.6	88, 90, 90,112,	82, 83, 96, 98,	42, 47,	5.5
116	66.5	60, 77, 84, 95,	54, 68, 93, 99,	75, 93,	1

Day 117. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

117a	66.5	66, 71, 72, 92,	15, 18, 20, 24,	3, 3,	80
122	48.5	66, 68, 72, 79,	25, 25, 25, 27,	4, 4,	55
124	69.3	90,108,111,123,	20, 22, 25, 55,	3, 3,	55
126	46.0	56, 65, 72,104,	28, 28, 30, 33,	4.5,6.5,	45
128	72.3	66, 68, 87, 91,	24, 26, 28, 29,	3, 3,	45
130	54.9	88, 91, 97, 97,	20, 22, 26, 26,	3.5,5.5,	45
132	69.1	54, 58, 65, 72,	22, 22, 26, 32,	3,4.5,	55
134	72.9	85, 91,106,111,	21, 24, 30, 32,	3, 4,	45
136	72.3	73, 76, 91,100,	21, 21, 22, 25,	3, 4,	60
138	72.4	77, 77, 84, 84,	22, 24, 25, 26,	3, 4,	50
140	67.4	55, 69, 69, 70,	24, 25, 25, 27,	3,4.5,	50

Table 38. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
142	67.7	68,	70,	89,	95,	25,	27,	27,	27,	4.5,	4.5,	45
144	57.4	94,	98,	98,	103,	35,	35,	40,	44,	5,	5,	30
146	56.5	73,	82,	85,	87,	28,	30,	30,	57,	5.5,	5.5,	35
148	43.4	85,	97,	97,	118,	58,	60,	62,	64,	4.5,	7,	15
150	44.2	84,	88,	97,	124,	43,	45,	50,	52,	7,	11,	20
152	43.2	65,	74,	79,	88,	58,	67,	70,	72,	9.5,	12,	10
154	43.1	86,	91,	95,	95,	53,	53,	72,	81,	7,	12,	15
156	49.8	86,	96,	98,	130,	48,	50,	50,	76,	6,	6,	15
158	64.0	66,	69,	80,	91,	44,	44,	59,	69,	16,	19,	8
160	60.7	73,	73,	99,	107,	56,	66,	71,	77,	21,	23,	6
162	66.8	91,	93,	97,	139,	67,	68,	72,	73,	33,	40,	3

Day 164. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

164	63.8	69,	72,	85,	91,	19,	19,	20,	20,	3,	3,	70
168	70.7	48,	51,	57,	58,	18,	21,	24,	24,	3,	3,	55
170	62.8	81,	81,	83,	83,	19,	21,	21,	26,	3,	4.5,	60
172	75.2	64,	88,	95,	96,	27,	27,	35,	41,	2.5,	2.5,	45
174	79.1	53,	54,	61,	95,	31,	33,	35,	39,	5,	9,	30
176	75.2	75,	77,	89,	128,	35,	39,	45,	48,	4,	5.5,	25
178	81.6	61,	62,	80,	92,	37,	39,	40,	54,	11,	22,	10
180	69.5	60,	68,	89,	115,	88,	91,	92,	94,	43,	84,	2
182	59.4	94,	101,	104,	128,	84,	96,	103,	140,	113,	156,	<1

Using Folin and Ciocalteu Reagent and the E.E.L. Colourimeter determination of phenol reacting substances was made during 2,4-D breakdown. They are recorded as 2,4-dichlorophenol.

Key to columns: H. Colourimeter reading in divisions.
I. Phenol reactors in perfusate (ppm.).

A.	H.	I.	A.	H.	I.
0	4.2	8.4	12	2.7	5.4
1	3.4	6.8	13	2.2	4.4
2	1.2	2.4	14	2.8	5.6
3	2.8	5.6	16	1.7	3.4
4	3.2	6.4	17	4.6	9.2
5	3.4	6.8	18	3.4	6.8
6	2.2	4.4	19	3.2	6.4
7	2.2	4.4	20	3.5	7.0
8	4.0	8.0	38	5.6	11.2
9	3.9	7.8	40	3.9	7.8
10	3.1	6.2			
11	3.4	6.8			

Table 38a. Direct perfusion of 2,4-D, followed by MCPA and this by 2,4-DCP.

Key to columns in table:
- A. Day of perfusion.
- B. Mean control root length (mm.).
- C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
- D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
- E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
- F. Indicated 2,4-D concentration in the perfusate (ppm.).
- G. Indicated MCPA concentration in the perfusate (ppm.).
- H. Colourimeter reading(E.E.L.) in divisions.
- I. Indicated 2,4-DCP concentration in the perfusate (ppm.).

Perfusion started on 31/5/51 with 60 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried October 1950,) and 250 ml. of 1,000 ppm. 2,4-D solution (common stock solution with Tables 41a, 41d, and 42,). Solution sampled before adding to the perfuser and after 2 hrs. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0b	40.0	88,	95,	98,	105,	28,	30,	35,	55,	5,	5,	600
0a	40.0	73,	75,	88,	93,	38,	40,	40,	53,	5,	7.5,	500
8	39.8	63,	63,	68,	78,	48,	63,	70,	73,	7.5,	10,	350
32	46.2	87,	91,	117,	124,	106,	110,	132,	141,	91,	98,	<10

Day 37. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

37a	40.7	76,	98,	98,	103,	44,	44,	52,	57,	5,	7.5,	450
39	37.7	75,	77,	93,	98,	48,	59,	64,	64,	8,	11,	300
41	36.7	68,	71,	71,	71,	49,	49,	49,	57,	8,	8,	300
43	35.2	74,	77,	80,	83,	68,	77,	77,	80,	26,	37,	110
45	42.8	84,	89,	98,	135,	98,	98,	100,	103,	105,	110,	<10

Day 49. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

49a	37.6	85,	98,	104,	109,	53,	59,	59,	61,	11,	13,	250
52	37.4	62,	72,	80,	80,	54,	56,	56,	83,	8,	8,	250
54	37.0	89,	89,	89,	97,	54,	60,	62,	68,	16,	30,	120
56	38.4	104,	104,	104,	107,	91,	97,	102,	117,	55,	60,	25

Day 59. 2.5 ml. of 1% stock 2,4-D solution added to the perfuser without draining to make the perfusate approximately 250 ml. of 1,000 ppm. Perfuser re-started, sampled after 1 hr.

59a	35.1	74,	91,	94,	100,	68,	71,	71,	86,	5.5,	5.5,	600
61	37.8	87,	98,	98,	111,	48,	50,	53,	58,	8,	5.5,	500
63	42.8	87,	87,	87,	89,	37,	37,	37,	47,	4.5,	4.5,	600
65	46.6	73,	73,	73,	88,	37,	41,	43,	49,	6.5,	8.5,	450
67	44.9	89,	91,	100,	102,	51,	51,	65,	74,	4.5,	9,	300
69	74.8	98,	103,	103,	110,	30,	39,	39,	45,	4,	5.5,	500

Table 38a. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
71	64.2	104,	109,	112,	115,	53,	58,	70,	73,	12,	19,	180
73	45.2	126,	128,	128,	142,	93,	93,	98,	118,	80,	87,	<10

Day 74. Perfuser drained and refilled with 150 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

74a	49.7	121,	123,	129,	139,	48,	48,	56,	74,	6,	14,	300
78	67.4	90,	96,	96,	110,	74,	74,	74,	82,	16,	28,	120
81	58.8	53,	65,	71,	77,	15,	17,	19,	20,	1.5,	3.5,	80
86	77.7	75,	76,	82,	87,	61,	66,	70,	71,	6.5,	9,	20
88	72.8	104,	107,	108,	117,	91,	92,	112,	118,	94,	94,	<10
90	74.8	76,	76,	79,	90,	82,	83,	84,	120,	88,	113,	<10
92	70.1	101,	103,	119,	120,	107,	109,	113,	126,	61,	67,	10

Day 93. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

93a	60.1	87,	102,	120,	127,	25,	25,	27,	35,	3.5,	5,	50
97	51.6	87,	99,	103,	124,	33,	33,	33,	35,	6,	6,	35
99	51.8	100,	102,	106,	126,	31,	33,	33,	35,	6,	6,	30
101	45.4	124,	135,	141,	157,	46,	53,	68,	84,	15,	18,	10
103	45.4	108,	128,	139,	152,	77,	77,	86,	95,	20,	24,	5
105	46.2	128,	130,	134,	141,	113,	119,	119,	130,	69,	74,	<1

Day 107. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

107a	49.2	78,	82,	82,	84,	26,	29,	33,	41,	4,	4,	50
112	76.3	92,	96,	98,	98,	37,	37,	53,	84,	14,	24,	15
114	68.8	84,	90,	90,	92,	99,	102,	108,	109,	102,	102,	<1

Day 116. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

116a	48.5	66,	68,	72,	85,	29,	29,	29,	29,	4,	6,	45
121	46.0	120,	130,	130,	137,	117,	126,	133,	137,	118,	146,	<1
123b	72.3	76,	82,	96,	103,	89,	91,	100,	109,	69,	96,	<1

Day 123. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

123a	72.3	55,	60,	60,	98,	22,	24,	26,	32,	4,	4,	55
127	69.1	87,	88,	90,	94,	90,	92,	101,	103,	82,	91,	<1

Day 129. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding to perfuser and after 1 hr. perfusion.

A.	H.	I.	A.	H.	I.	A.	H.	I.
129ba	100	200,	133	20.5	41,	136alhr	78	156,
129alhr	56	112,	134	20.4	41,	137	48.5	97,
130	32.7	65.5,	135	15	30,	138	42	84,
131	28.7	57.5,	136b	9.2	18.5,	139	Not determined.	
132	24.5	49,	136ba	98	196,	140	37	74,

Table 39. Perfusion of stored, previously enriched, soil with 2,4-D, followed by ∝-4-CPP.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length.(mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Indicated 2,4-D concentration in the perfusate (ppm.).
G. Indicated ∝-4-CPP concentration in the perfusate (ppm.).

Perfusion started on 14/6/53 with 50 gm. of soil (This soil had been enriched to 2,4-D in a large perfuser, Table 31, then stored wet in the perfuser tube for 1 year. It was then dried and sieved in the usual way.) and 250 ml. of sterile 2,4-D solution. First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	91.7	72,	77,	77,	84,	22,	23,	24,	25,	3.5,	3.5,	95
6	103.4	82,	86,	88,	88,	30,	32,	43,	44,	5,	6,	65
8	101.7	96,	99,	99,	102,	83,	84,	85,	100,	85,	90,	<1

Day 10. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

10a	100.1	87,	88,	90,	94,	23,	25,	28,	29,	3,	3,	85
12	98.1	96,	99,	106,	109,	106,	108,	110,	117,	98,	102,	<1

Day 14. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

14a	102.9	79,	82,	82,	89,	24,	27,	28,	31,	3,	4,	80
16	102.7	89,	91,	97,	102,	95,	96,	103,	110,	99,	101,	<1

Day 18. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

18a	97.5	83,	86,	92,	105,	25,	28,	29,	35,	3,	4,	75
20	99.3	99,	104,	105,	108,	94,	96,	98,	102,	94,	106,	<1

Day 22. Perfuser drained and refilled with 250 ml. of 100 ppm. ∝-4-CPP solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
22a	105.4	96,	96,	97,	103,	34,	36,	52,	55,	17,	19,	100
25	95.2	92,	96,	97,	101,	39,	40,	50,	58,	14,	19,	65
28	99.6	91,	94,	99,	102,	43,	44,	50,	65,	19,	20,	40
31	99.8	99,	99,	101,	103,	82,	83,	85,	98,	28,	34,	20
34	95.9	100,	101,	102,	110,	96,	97,	101,	110,	51,	55,	8

Table 39. continued.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	G.
37	102.9	99, 99,102,107,	99,101,110,111,	47, 48,	6
40	102.6	99, 99,103,111,	101,101,107,107,	61, 63,	5
43	103.4	91, 91, 99, 99,	92, 93, 93, 98,	82, 85,	2
46	87.7	90,100,103,116,	97, 98,103,110,	77, 95,	1.5
49b	102.3	98, 99,102,107,	99,102,104,106,	76, 94,	1.5

Day 49. Perfuser drained and refilled with 250 ml. of 100 ppm. ∝-4-CPP solution. Perfuser re-started, sampled after 1 hr.

49a	97.0	87, 87, 89,104,	47, 49, 57, 58,	19, 21,	80
52	97.1	99,102,103,105,	80, 91, 93, 93,	39, 49,	12.5
55	93.9	96, 97,102,106,	93, 94,100,101,	52, 52,	8
58	99.8	93, 96, 98, 99,	101,102,103,104,	38, 43,	9
61	99.1	96, 99,103,105,	97,100,100,111,	60, 70,	5
67	105.1	98, 98,100,103,	86, 87, 87, 96,	69, 70,	4

Table 39a. Perfusion of stored, previously enriched, soil with 2,4-D, followed by ∝-4-CPP.

Key to columns in table: as in Table 39, above.
Details of perfusion set-up: as in Table 39, above.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
0	91.7	77, 77, 78, 78,	21, 22, 23, 23,	3.5,3.5,	100
6	103.4	85, 86, 88, 96,	30, 31, 31, 40,	4, 6,	65
8	101.7	92, 95, 96,101,	97,101,102,111,	106,111,	<1

Day 10. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

10a	100.1	91, 91, 92, 95,	21, 23, 23, 25,	3, 4,	95
12	98.1	96, 99,100,105,	100,101,102,107,	109,111,	<1

Day 14. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

14a.	102.9	67, 75, 76, 85,	20, 24, 27, 30,	2, 3,	95
16	102.7	93, 97, 98,103,	86, 88, 90, 90,	84, 86,	1

Day 18. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

18a	97.5	90, 90, 93, 98,	24, 24, 24, 26,	3, 3,	90
20	99.3	96, 99,105,114,	82, 88, 92, 99,	81, 89,	1

Table 39a. continued.

Day 22. Perfuser drained and refilled with 250 ml. of 100 ppm. ∝-4-CPP solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
22a	105.4	86,	87,	87,	90,	41,	43,	44,	52,	19,	19,	80
25	95.2	87,	90,	94,	101,	41,	41,	41,	42,	17,	19,	80
28	99.6	96,	96,	97,	99,	45,	50,	58,	60,	15,	17,	55
31	99.8	102,	104,	109,	110,	77,	81,	82,	83,	19,	21,	20
34	95.9	100,	100,	102,	102,	90,	92,	99,	103,	37,	45,	10
37	102.9	97,	98,	103,	105,	92,	94,	94,	98,	52,	61,	5
40	102.6	94,	95,	95,	100,	95,	95,	96,	97,	52,	55,	5
43	103.4	95,	96,	99,	102,	91,	96,	99,	102,	53,	60,	5
46b	96.3	100,	101,	102,	104,	91,	93,	100,	103,	51,	54,	5

Day 46. Perfuser drained and refilled with 250 ml. of 100 ppm. ∝-4-CPP solution. Perfuser re-started, sampled after 1 hr.

46a	87.7	97,	97,	101,	101,	60,	64,	69,	71,	21,	28,	30
49	97.0	88,	91,	94,	97,	79,	84,	84,	94,	33,	35,	12
52	97.1	107,	108,	109,	110,	94,	97,	100,	103,	68,	70,	2.5
55	93.9	98,	99,	101,	101,	90,	91,	93,	108,	65,	71,	2.5
58	99.8	95,	100,	106,	115,	93,	94,	94,	100,	63,	68,	3
61	99.1	99,	100,	100,	107,	96,	96,	99,	101,	64,	67,	3
67	105.1	90,	90,	93,	103,	93,	96,	98,	99,	75,	86,	1.5

Table 40. Perfusion of stored, previously enriched, soil with 2,4-D and followed by ∝-2,4-DCPP.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length.(mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Longest roots at nominal concentration of 10 ppm. as % m.c.
G. Indicated 2,4-D concentration in the perfusate (ppm.).
H. Indicated ∝-2,4-DCPP concentration in the perfusate (ppm.).

Perfusion started on 1/6/53 with 50 gm. of soil (This soil had been enriched to 2,4-D in a large perfuser, Table 31, then stored wet in the perfuser tube for 1 year. It was then dried and sieved in the usual way.) and 250 ml. of sterile 2,4-D solution. First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
0	93.9	78,	80,	92,	102,	17,	19,	21,	29,	3,	3,	100
3	92.3	68,	79,	80,	91,	16,	17,	20,	21,	2,	3,	100
6	98.6	88,	88,	93,	104,	21,	21,	28,	32,	3,	4,	85
9	95.2	92,	93,	96,	102,	87,	88,	92,	103,	98,	101,	<1
Day 12. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.												
12a	94.9	76,	78,	81,	87,	26,	29,	29,	32,	2,	3,	110
15	94.9	92,	93,	102,	106,	100,	104,	105,	106,	96,	97,	<1
Day 18. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.												
18a	94.4	87,	91,	93,	95,	20,	20,	23,	25,	3,	5.5,	100
20	104.1	98,	98,	98,	111,	92,	96,	98,	98,	86,	92,	<1
22b	98.1	100,	101,	105,	105,	105,	105,	107,	110,	96,	105,	<1
Day 22. Perfuser drained and refilled with 250 ml. of 100 ppm. ∝-2,4-DCPP solution. Perfuser re-started, sampled after 1 hr.												

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	H.
22a	98.1	25,	28,	32,	41,	5,	5,	6,	6,	1,	1,	90
28	98.1	29,	29,	32,	39,	4,	6,	7,	9,	1,	2,	100
34	96.6	25,	26,	27,	28,	5,	5,	6,	7,	1,	2,	110
40	98.6	26,	27,	28,	28,	5,	6,	7,	10,	1,	2,	110
46	95.9	27,	28,	32,	34,	5,	6.5,	6.5,	9.5,	1,	2,	95
52	99.7	31,	32,	32,	38,	7,	8,	10,	10,	2,	2,	90

Table 40a. Perfusion of stored, previously enriched, soil with 2,4-D and followed by ∝-2,4-DCPP.

Key to columns in table: as in Table 40, above.
Details of perfusion set-up: as in Table 40, above.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
0	93.9	78,	80,	92,	102,	17,	19,	21,	29,	3,	3,	105
3	92.3	69,	72,	80,	82,	14,	19,	21,	23,	2,	3,	105
6	98.6	88,	92,	92,	95,	24,	27,	30,	36,	3,	3,	70
9	95.2	95,	101,	102,	105,	103,	103,	104,	104,	94,	97,	<1
Day 12. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.												
12a	94.9	77,	78,	84,	86,	22,	25,	29,	29,	2,	2,	100
15	94.9	99,	100,	101,	103,	99,	102,	104,	106,	90,	108,	<1
Day 18. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.												
18a	94.4	93,	95,	97,	107,	28,	29,	29,	43,	4,	5.5,	70
20	104.1	88,	92,	93,	99,	95,	99,	100,	101,	86,	100,	<1
22b	98.1	93,	94,	105,	109,	102,	113,	115,	119,	94,	102,	<1
Day 22. Perfuser drained and refilled with 250 ml. of 100 ppm. ∝-2,4-DCPP solution. Perfuser re-started, sampled after 1 hr.												
A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	H.
22a	98.1	31,	33,	39,	40,	6,	7,	7,	9,	1,	2,	80
28	98.1	25,	25,	29,	32,	4,	5,	6,	7,	1,	2,	105
34	96.6	25,	33,	34,	37,	4,	5,	5,	7,	1,	1,	100
40	98.6	20,	20,	20,	21,	5,	6,	7,	10,	1,	2,	90
46	95.9	29,	30,	32,	37,	4,	5,	5,	6.5,	1,	1,	100
52	99.3	27,	27,	29,	29,	6,	7,	7,	9,	1,	2,	110

Table 41. Direct perfusion of 2,4-D, followed by 2,4-DCP.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Indicated 2,4-D concentration in the perfusate (ppm.).
G. Colourimeter reading (E.E.L. instrument,) in divisions.
H. Indicated 2,4-DCP concentration in the perfusate (ppm.).

Perfusion started on 16/2/51 with 80 gm. of soil (1 to 4 mm., Sussex Lodge soil, dried September 1950,) and 250 ml. of 1,000 ppm. 2,4-D solution. Sampled before adding to perfuser and after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0ba	29.2	58,	68,	82,	99,	45,	45,	48,	51,	10,	10,	400
0alhr	29.2	79,	79,	79,	96,	51,	55,	58,	58,	7,	7,	500
4	38.0	61,	63,	68,	82,	26,	29,	32,	37,	8,	8,	500
11	22.6	89,	93,	119,	124,	40,	58,	67,	84,	9,	13,	250
14	31.1	58,	77,	96,	112,	35,	35,	39,	42,	0,	0,	550
17	30.0	80,	83,	103,	113,	30,	37,	37,	40,	7,	10,	450
19	30.1	83,	83,	87,	93,	33,	40,	43,	50,	6.5,	10,	450
21	37.6	85,	101,	106,	112,	40,	45,	51,	72,	10,	13,	250
23	23.5	81,	89,	98,	158,	72,	89,	89,	106,	98,	102,	<10
25	35.9	61,	70,	95,	97,	89,	97,	100,	109,	78,	87,	<10

Day 27. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1/4 hr.

27a	30.0	60,	70,	70,	77,	23,	30,	33,	50,	3,	10,	60
28	30.0	57,	60,	77,	87,	73,	73,	90,	97,	7,	7,	45
29	29.3	41,	58,	65,	109,	120,	120,	120,	123,	75,	85,	1
30	35.4	91,	94,	96,	113,	105,	119,	119,	127,	85,	113,	<1

Day 31. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

A.	G.	H.	A.	G.	H.	A.	G.	H.
0ba.	3.5	7,	11	4.3	9,	23	3.0	6,
0alhr.	3.8	8,	12	3.0	6,	24	2.9	6,
1	4.8	10,	13	4.8	10,	25	4.1	8,
2	6.0	12,	14	6.0	12,	26	3.9	8,
3	6.4	13,	15	4.6	9,	27b.	3.9	8,
4	6.0	12,	16	4.5	9,	27a.	1.3	3,
5	3.7	8,	17	4.5	9,	28	3.5	7,
6	3.8	8,	18	4.6	9,	29	3.6	7,
7	3.8	8,	19	4.1	8,	30	2.4	5,
8	3.4	7,	20	4.3	9,	31b.	2.8	6,

Table 41. continued.

A.	G.	H.	A.	G.	H.	A.	G.	H.
9	3.8	8,	21	4.0	8,			
10	4.3	9,	22	4.2	8,			

Day 31. Drained and refilled with 250 ml. of 200 ppm. 2,4-DCP.

31ba.	100	200,	32	36.2	72,	46	3.6	7,
31alhr.	51.5	103,	33	31	62,	47b.	3.9	8,

Day 47. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Perfuser re-started, first sample after 1 hr.

47alhr.	50	100,	52	31.6	63,	57	18.6	37,
48	44	88,	53	25.2	50,	58	15.8	32,
49	34.4	69,	54	26.1	52,	59	9.1	18,
50	35.5	71,	55	23.5	47,	60b.	6.8	14,
51	35.0	70,	56	22.0	44,			

Day 60. Perfuser drained and refilled with 250 ml. of solution containing 200 ppm. 2,4-DCP and 0.01% sodium azide. First sample after 1 hr. perfusion.

60alhr.	60	120,	74	36.4	73,	88	23.1	46,
61	48.5	97,	75	36.7	74,	89	22.8	46,
62	46.5	93,	76	34.4	69,	90	20.7	41,
63	46.3	93,	77	33.2	66,	91	20.0	40,
64	44.1	88,	78	33.6	67,	92	17.0	34,
65	44.0	88,	79	32.0	64,	93	15.2	30,
66	42.9	86,	80	32.4	65,	94	10.4	21,
67	41.7	83,	81	29.5	59,	95	8.0	16,
68	42.3	85,	82	29.1	58,	96	9.2	18,
69	39.6	80,	83	28.8	58,	97	9.0	18,
70	41.0	82,	84	28.4	57,	98	10.8	22,
71	36.8	74,	85	27.0	54,	99	9.0	18,
72	33.1	66,	86	24.0	48,	100	8.9	18,
73	33.9	68,	87	24.2	48,			

Table 41a. Direct perfusion of 2,4-D, followed by 2,4-DCP.

Key to columns in table: as in Table 41, above.

Perfusion started on 31/5/51 with 60 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried October 1950,) and 250 ml. of 1,000 ppm. 2,4-D solution (common sterile bulk solution used for this perfuser and those of Tables 38a, 41d, and 42,). Solution sampled before adding to the perfuser and after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0ba	40.0	88,	95,	98,	105,	28,	30,	35,	55,	5,	5,	650
0alhr	40.0	73,	75,	83,	85,	28,	33,	38,	45,	5,	5,	650
8	39.8	80,	80,	83,	88,	40,	43,	50,	63,	5,	5,	500
32	43.7	108,	110,	110,	119,	96,	108,	110,	123,	94,	101,	<10

Day 37. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

37a	40.7	89,	98,	103,	106,	49,	53,	54,	76,	5,	5,	500
39	37.7	72,	75,	85,	93,	40,	43,	43,	48,	8,	11,	400
41	36.7	82,	82,	87,	90,	57,	60,	60,	65,	5.5,	8,	400
43	35.2	94,	94,	94,	100,	60,	65,	65,	74,	8.5,	12,	300
45	42.8	77,	87,	87,	89,	87,	89,	89,	98,	56,	58,	25

Day 49. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

49a	37.6	74,	80,	88,	91,	51,	51,	56,	69,	8,	11,	400
52	37.4	51,	59,	59,	70,	43,	51,	54,	59,	8,	11,	450
54	37.0	84,	87,	106,	106,	70,	73,	76,	79,	24,	27,	100
56	38.4	78,	78,	81,	89,	73,	78,	81,	99,	47,	59,	30

Day 59. 25 ml. of 1% 2,4-D solution added to the perfuser without draining, making the final perfusate approximately 250 ml. of 1,000 ppm. 2,4-D. Sampled after 1 hr. perfusion.

59a	35.1	83,	83,	86,	108,	54,	54,	60,	74,	5.5,	5.5,	450
61	37.8	74,	77,	85,	85,	45,	45,	45,	53,	5.5,	8,	400
63	42.8	75,	84,	87,	96,	47,	54,	61,	61,	4.5,	7,	400
65	46.6	64,	77,	82,	88,	47,	49,	49,	52,	6.5,	8.5,	400
67	44.9	83,	91,	94,	105,	42,	51,	54,	56,	4.5,	4.5,	400
69	74.8	104,	107,	107,	118,	25,	33,	37,	43,	5.5,	5.5,	650
71	64.2	90,	104,	106,	125,	28,	28,	30,	34,	4.5,	4.5,	700
73	45.2	80,	82,	89,	91,	24,	27,	29,	38,	6.5,	6.5,	700
75	49.7	91,	91,	93,	97,	30,	32,	38,	40,	6,	10	600
77	48.9	72,	78,	90,	96,	43,	45,	55,	55,	6,	8,	450
79	67.3	86,	94,	98,	114,	34,	39,	40,	46,	4.5,	7.5,	500
81	58.8	89,	92,	99,	104,	32,	34,	36,	46,	7,	12,	550
83	70.0	90,	91,	93,	114,	36,	36,	44,	46,	5.5,	9,	500
85	70.0	91,	94,	94,	96,	73,	73,	81,	86,	16,	21,	150
87b	67.1	104,	106,	121,	131,	107,	118,	119,	127,	109,	115,	<10

Table 41a. continued.

Day 87. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
87a	67.1	70,	91,	95,	113,	27,	28,	28,	28,	10,	14,	750
91	62.6	97,	114,	125,	131,	26,	27,	30,	32,	5.5,	8,	750
93	58.6	89,	91,	99,	106,	26,	27,	33,	48,	8.5,	12,	550
95	60.1	105,	127,	132,	135,	70,	77,	80,	92,	8.5,	14,	250
97	61.7	110,	112,	118,	136,	107,	110,	110,	118,	110,	117,	<10

Day 99. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding to the perfuser and after 1 hr. perfusion.

A.	G.	H.	A.	G.	H.	A.	G.	H.
99ba.	87,	174,	99alhr.	37.2	74,	100b.	8.6	17,

Day 100. Drained and refilled with 250 ml. of 200 ppm. 2,4-DCP.

100ba.	102	204,	100alhr.	57	114,	101b.	5	10,

Day 101. Drained and refilled with 250 ml. of 200 ppm. 2,4-DCP.

101ba.	96	192,	103	27	54,	106	22	44,
101alhr.	56	112,	104	24.7	49	107b.	17.1	34,
102	35	70,	105	23.2	46,			

Day 107. Drained and refilled with 250 ml. of 200 ppm. 2,4-DCP.

107ba.	102	204,	115	37.6	75,	124	30.5	61,
107alhr.	58.5	117,	116	41	82,	125	29.4	59,
108	46	92,	117	38.5	77,	126	29	58,
109	47	94,	118	38.5	77,	127	26.1	52,
110	48.2	96,	119	36.5	73,	128	23.5	47,
111	43.5	87,	120	33.4	67,	129	17.8	36,
112	43	86,	121	36.4	73,	130	13.2	26,
113	43	86,	122	35.9	72,	131b.	9	18,
114	42	84,	123	29.8	60,			

Day 131. Drained and refilled with 250 ml. of 200 ppm. 2,4-DCP.

131ba.	117	234,	136	45.5	91,	142	40	80,
131alhr.	59.5	119,	137	43.6	87,	143	/	/,
132	48	96,	138	41	82,	144	36.2	72,
133	37.5	75,	139	/	/,	145	/	/,
134	45	90,	140	42	84,	146	26.5	53,
135	40.8	82,	141	/	/,	147b.	16	32,

Day 147. Drained and refilled with 250 ml. of 200 ppm. 2,4-DCP.

147ba.	95	190,	151	47	94,	155	33.4	67,
147alhr.	61	122,	152	41.6	83,	156	/	/,
148	46	92,	153	38.5	77,	157	24.5	49,
149	47.7	95,	154	38.5	77,	159b	6.7	13,

Table 41a. continued.

Day 159. Drained and refilled with 250 ml. of 200 ppm. 2,4-DCP.

A.	G.	H.	A.	G.	H.	A.	G.	H.
159ba.	112	224,	162	44	88,	166	36	72,
159alhr.	62.5	125,	163	40.1	80,	167	27.3	55,
160	52	104,	164	42	84,	168	13.7	27,
161	46.2	92,	165	38	76,	169b.	5.8	12,

Day 169. Drained and refilled with 250 ml. of 200 ppm. 2,4-DCP.

169ba.	102	204,	171	42	84,	174	24.1	48,
169alhr.	66	132,	172	36.5	73,	175b.	13	26,
170	46.5	93,	173	33	66,			

Day 175. Drained and refilled with 250 ml. of 200 ppm. 2,4-DCP.

175ba.	100	200,	177	37.2	74,	180b.	5.2	10,
175alhr.	77	154,	178	18.4	37,			
176	46	92,	179	7.5	15,			

Day 180. Drained and refilled with 250 ml. of 200 ppm. 2,4-DCP.

180ba.	100	200,	181	41.5	83,	183	21.1	42,
180alhr.	68	136,	182	32	64,	184b.	7	14,

Day 184. Drained and refilled with 250 ml. of 200 ppm. 2,4-DCP.

184ba.	105	210,	185	38.8	78,	187	23	46,
184alhr.	56	112,	186	38.6	77,	188b.	6.5	13,

Day 188. Perfuser not drained, but 10 ml. of 1% 2,4-D solution added to make the perfusate approximately 250 ml. of 400 ppm. Sampled after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
188	70.7	32,	42,	46,	59,	6,	7.5,	8.5,	8.5,	1.5,	1.5,	450
190	76.3	51,	51,	52,	59,	8,	9,	10,	13,	1.5,	1.5,	300
193	75.4	67,	73,	80,	84,	14,	14,	15,	16,	2.5,	4,	180
196	71.4	65,	65,	66,	96,	18,	19,	21,	23,	4,	5.5,	120
199	60.3	88,	95,	95,	96,	18,	20,	22,	30,	5,	6.5,	65
213	73.3	85,	89,	93,	108,	86,	90,	98,	114,	74,	90,	1
216b	81.3	99,	100,	100,	119,	67,	78,	105,	113,	90,	95,	<1

Day 216. 5 ml. of 1% 2,4-D solution and water added, without draining, to make the perfusate approximately 250 ml. of 200 ppm. Sampled after 1 hr. perfusion.

216a	70.8	42,	44,	48,	52,	8.5,	10,	13,	17,	2.5,	2.5,	220
219	77.7	73,	80,	90,	107,	14,	17,	23,	23,	2,	2,	120
222	72.0	81,	84,	100,	110,	60,	72,	77,	86,	11,	21,	15
224b	59.0	100,	104,	110,	114,	112,	116,	120,	124,	122,	124,	<1

Day 224. 5ml. of 1% 2,4-D solution added without draining, to give approx. 250 ml. of 200 ppm. 2,4-D. Sampled after 1 hr.

224a	59.0	68,	75,	78,	114,	17,	17,	19,	25,	1.5,	2.5,	140
228	59.7	74,	83,	95,	97,	70,	76,	82,	95,	25,	28,	10
230	59.1	123,	128,	139,	149,	125,	137,	161,	176,	120,	137,	<1

Table 41b. Transferred adaptation to 2,4-D, followed by 2,4-DCP.

Key to columns in table: as in Table 41.

Perfusion started on 20/8/51 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried June 1951,) and 250 ml. of 1,000 ppm. 2,4-D solution prepared from the "active" perfusate drained from a 2,4-D enriched perfuser (Table 38,) on 20/8/51. First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	58.8	60,	60,	60,	63,	19,	24,	24,	44,	5,	5,	950
5	77.7	63,	64,	64,	75,	17,	17,	18,	22,	2.5,	4,	1,200
7	67.1	57,	58,	63,	70,	18,	19,	19,	24,	6,	7.5,	1,200
9	74.8	59,	72,	74,	84,	17,	19,	21,	21,	2.5,	4,	1,100
11	70.1	79,	89,	90,	112,	27,	27,	29,	43,	6,	9,	750
13	56.4	96,	98,	104,	108,	92,	99,	103,	124,	92,	128,	<10
15	60.1	130,	132,	133,	152,	110,	110,	113,	120,	125,	130,	<10

Day 17. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

17a	51.6	72,	80,	107,	112,	25,	25,	27,	35,	4,	6,	850
21	45.4	115,	117,	121,	126,	104,	104,	110,	117,	106,	113,	<10

Day 25. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

25a	56.0	61,	65,	70,	100,	23,	23,	25,	30,	3.5,	7,	950
29	66.5	69,	71,	75,	86,	54,	60,	69,	71,	75,	92,	<10

Day 32. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding to the perfuser and after 1 hr.

A.	G.	H.	A.	G.	H.	A.	G.	H.
32ba	114	228,	39	32	64,	47	22.4	45,
32alhr	68.5	137,	40	32	64,	48	21.4	43,
33	48	96,	41	31	62,	49	21.4	43,
34	43.5	87,	42	25	50,	50	22.2	44.5,
35	39.6	79,	43	25.5	51,	51	21.9	44,
36	33.5	67,	44	26.2	52.5,	52	20	40,
37	36	72,	45	26	52	53	19.2	38.5,
38	34	68,	46	25.3	50.5,	54	16	32.0,
						55b	15	30,

Day 55. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding to the perfuser and after 1 hr. perfusion.

55ba	98	196,	57	55	110,	60	55	110,
55alhr	76	152,	58	56.5	113,			
56	57.5	115,	59	57	114,			

Table 41c. Transferred adaptation to 2,4-D, followed by 2,4-DCP.

Key to columns in table: as in Table 41, above.

Perfusion started on 18/8/51 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried June 1951,) and 250 ml. of 1,000 ppm. 2,4-D solution prepared from the "active" perfusate drained from an enriched perfuser, enriched to 2,4-D and 2,4-DCP, (Table 72e,) on 18/8/51. First sample taken after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	67.3	89,	97,	101,	103,	31,	33,	33,	34,	6,	6,	600
3	58.7	104,	104,	112,	113,	34,	34,	39,	49,	3.5,	5,	600
5	70.0	114,	116,	120,	134,	89,	90,	99,	117,	16,	20,	100
7	77.7	107,	110,	111,	121,	110,	112,	113,	122,	95,	103,	<10

Day 8. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

8a	67.2	86,	89,	91,	97,	28,	30,	34,	36,	1.5,	3,	700
12	62.6	106,	107,	112,	114,	59,	69,	75,	89,	16,	24,	150
14b	56.4	117,	119,	124,	154,	112,	114,	117,	137,	112,	135,	<10

Day 14. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

14a	60.1	108,	118,	122,	133,	22,	25,	27,	30,	5,	7,	800
18	51.6	95,	99,	103,	115,	100,	130,	136,	140,	89,	93,	<10
20	51.8	95,	97,	100,	108,	104,	108,	110,	112,	99,	99,	<10

Day 21. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. This solution sampled before adding to the perfuser and after 1 hr. perfusion.

A.	G.	H.	A.	G.	H.	A.	G.	H.
21ba	102	204,	22	45	90,	24	25	50,
21alhr	74	148,	23	36	72,	25b	6.8	14,

Day 25. Perfuser drained and refilled with 250 ml. of approximately 400 ppm. 2,4-DCP solution. Solution sampled before adding to the perfuser and after 1 hr. perfusion.

25ba	2x97	388,	36	59	118,	48	47	94,
25alhr	2x74	296,	37	69.5	139,	49	49.5	99,
26	2x48.6	196,	38	57	114,	50	49	98,
27	95	190,	39	67	134,	51	46.3	93,
28	91	182,	40	59.5	119,	52	37.4	75,
29	84	168,	41	57	114,	53	41.4	83,
30	78	156,	42	55	110,	54	42	84,
31	72	144,	43	58	116,	55	44	88,
32	74	148,	44	53.5	107,	56	36.4	73,
33	75	150,	45	43.2	86,	57b	22.6	45,
34	62	124,	46	55	110,			
35	71	142,	47	55	110,			

Table 41c. continued.

Day 57. Perfuser drained and refilled with 250 ml. of distilled water. Sampled after 1 hr. perfusion.

A.	G.	H.	A.	G.	H.	A.	G.	H.
57alhr	6.0	12,	62	3.5	7,	69	3.5	7,
58	8.5	17,	63	/	/,	70	/	/,
59	5.0	10,	64	/	/,	71	4.0	8,
60	2.8	5.5,	65	5.0	10,	72	/	/,
61	4.5	9,	66	/	/,	73b	5.0	10,
			67	3.0	6,			
			68	2.8	5.5,			

Day 73. Perfuser drained and refilled with 250 ml. of approximately 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr. perfusion.

73ba	108	216,	75	53	106,	79	13	26,
73alhr	70	140,	77	44.1	88,	80b	3.7	7.5,

Day 80. Perfuser drained and refilled with 250 ml. of approximately 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr. perfusion.

80ba	112	224,	83	53	106,	86	33.5	67,
80alhr	71	142,	84	48	96,	87	12.3	25,
81	65	130,	85	46.3	93,	88b	4.4	9,
82	52	104,						

Day 88. Perfuser drained and refilled with 250 ml. of approximately 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr. perfusion.

88ba	107	214,	91	52.2	104,	95	23.8	48,
88alhr	79.5	159,	92	41.5	83,	96b	6	12,
89	56.5	113,	93	43.5	87,			
90	58	116,	94	39	78,			

Day 96. Perfuser drained and refilled with 250 ml. of approximately 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr. perfusion.

96ba	100	200,	98	51	102,	101	13.5	27,
96alhr	72	144,	99	44	88,	102b	5.3	11,
97	54	108,	100	28	56,			

Day 102. Perfuser drained and refilled with 250 ml. of approximately 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr. perfusion.

102ba	104	208,	106	54	108,	111	19.8	40,
102alhr	80	160,	107	48.6	97,	112	15.6	31,
103	59	118,	108	45	90,	113	12.5	25,
104	63	126,	109	41.5	83,	114b	9	18,
105	59.6	119,	110	34.3	69,			

Table 41c. continued.

Day 114. Perfuser drained and refilled with 250 ml. of approximately 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr. perfusion.

A.	G.	H.	A.	G.	H.	A.	G.	H.
114ba	107	214,	119	46	92,	125	12	24,
114alhr	76.5	153,	120	38.4	77,	126	/	/,
115	62	124,	121	33.4	67,	134	0.5	1,
116	51.4	103,	122	28.3	57,	135	1.0	2,
117	49	98,	123	24	48,	136	0.5	1,
118	51	102,	124	14	28,	137	0	0,
						138	0.8	1.5,

Day 139. Perfuser drained and refilled with 250 ml of approximately 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr. perfusion.

139ba	100	200,	141	53	106,	144	31.5	63,
139alhr	83	166,	142	46.1	92,	145	22.4	45,
140	55.5	111,	143	38	76,	146b	4.8	10,

Day 146. Perfuser drained and refilled with 250 ml. of approximately 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr. perfusion.

146ba	107	214,	149	53	106,	153	27.2	54,
146alhr	77.5	155,	150	48.6	97,	154	23.8	48,
147	55	110,	151	43	86,	155b	13	26,
148	48.8	98,	152	34.2	68,			

Day 155. Perfuser drained and refilled with 250 ml. of approximately 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr. perfusion.

155ba	92.5	185,	157	45	90,	159	15.3	31,
155alhr	67	134,	158	36	72,	160	6	12,
156	49	98,						

Table 41d. Direct perfusion of 2,4-D, followed by 2,4-DCP.

Key to columns in table: as in Table 41, above.

Details of perfusion set-up: as for Table 42, for which, along with Table 41a, a common, bulk, sterile solution of 1,000 ppm. 2,4-D was used. Perfusion started on 31/5/51.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
0ba	40.0	88, 95, 98,105,	28, 30, 35, 55,	5, 5,	650
0a2hr	40.0	68, 70, 78, 83,	33, 43, 43, 45,	7.5, 10,	400
8	40.0	63, 73, 73, 75,	43, 43, 45, 53,	7.5, 13,	400
32	46.2	95,108,108,130,	91,102,102,106,	93,102,	<10

Day 37. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

37a	40.7	71, 71, 74, 94,	44, 47, 47, 54,	5,7.5,	500
39	37.7	85, 85, 96,101,	50, 59, 59, 61,	5.5, 8,	400
41	36.7	85, 93, 96,101,	55, 55, 57, 63,	8, 8,	350
43	35.2	80, 85,102,105,	80, 83, 91, 94,	26, 37,	80
45	42.8	84, 89, 96, 98,	105,108,117,128,	84, 87,	<10

Day 49. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

49a	37.6	80, 88, 93,101,	53, 59, 59, 61,	8, 19,	400
52	37.4	83, 83, 88,107,	54, 56, 59, 64,	8, 16,	350
54	37.0	84, 87,100,106,	60, 60, 62, 62,	8, 16,	300
56	38.4	73, 78, 81,107,	73, 76, 78, 81,	13, 21,	150
58	33.6	81, 86, 89, 95,	95,107,113,113,	104,131,	<10

Day 62. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 2 hr.

62a	42.8	77, 91, 94,108,	49, 51, 51, 56,	7, 7,	450
64	46.6	82, 90, 93, 97,	39, 41, 41, 47,	6.5,6.5,	500
66	40.1	72, 75, 90,102,	45, 50, 55, 75,	7.5,7.5,	500
68	43.5	74, 76, 83, 90,	46, 48, 48, 58,	7, 9,	500
70	64.2	90,109,110,112,	37, 39, 45, 48,	9.5, 11,	450
72	62.2	97,116,122,124,	64, 71, 85, 85,	11, 15,	200

Day 74. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

74a	45.2	80,117,122,126,	20, 24, 31,38,	4.5,6.5,	750
78	67.4	79, 88, 92,114,	37, 37, 42, 50,	9, 9,	500
81	58.8	107,112,112,117,	41, 44, 48, 49,	10,13 ,	300
83	70.0	101,107,110,121,	64, 83,100,107,	17, 19,	100
85	70.0	100,110,114,139,	91, 93, 96,124,	91, 97,	<10

Day 87. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

87	67.1	79, 91,101,116,	25, 27, 27, 30,	6,7.5,	800
91	62.6	114,114,114,126,	29, 34, 46, 54,	5,6.5,	500

Table 41d. continued.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
93	58.6	92, 97,113,116,	26, 29, 29, 31,	3.5, 7,	700
95	60.1	92, 98,103,123,	33, 37, 38, 40,	6.5,8.5,	500
97	61.7	89,114,120,136,	29, 31, 44, 58,	8,9.5,	450
99	51.8	79, 85,100,102,	33, 33, 48, 56,	7.5, 14,	300
101	42.3	92, 95,130,152,	59, 64, 66, 76,	31, 31,	100
103	45.4	95,104,119,132,	128,135,135,144,	106,106,	10
105b	46.2	100,104,106,119,	98,102,102,147,	108,124,	10

Day 105. Perfuser drained and refilled with 250 ml. of 1,000 ppm 2,4-D solution. Perfuser re-started, sampled after 1 hr.

105a	46.2	85, 87, 89, 95,	30, 37, 43, 48,	6.5,6.5,	500
109	62.6	72, 83, 86, 96,	27, 29, 30, 37,	6.5, 11,	700
111	66.5	59, 65, 83, 96,	27, 32, 35, 41,	6, 6,	600
113	49.3	95,108,114,114,	39, 46, 47, 63,	10, 10,	350
115	57.5	87, 90, 94,110,	64, 66, 70, 84,	12, 14,	200
117	48.5	101,110,112,114,	87, 89, 93,132,	101,112,	10
119	73.0	67, 85, 89, 93,	72, 79, 82, 93,	100,103,	10

Day 119. Perfuser drained and refilled with 250 ml. of approximately 200 ppm. 2,4-DCP solution. Solution sampled before adding to the perfuser and after 1 hr. perfusion.

A.	G.	H.	A.	G.	H.	A.	G.	H.
119ba	96	192,	119alhr	74	148,	120b	19.5	39,

Day 120. Perfuser drained and refilled with 250 ml. of approximately 200 ppm. 2,4-DCP solution. Solution sampled before adding to the perfuser and after 1 hr. perfusion. The sample taken on the following day was 16 hrs. after the refill.

120ba	105	210,	129	32.6	65,	139	/	/,
120alhr	68	136,	130	30.5	61,	140	24.5	49,
121a16hr	45	90,	131	28.8	57.5,	141	/	/,
122	46.6	93,	132	29	58,	142	22	44,
123	42.5	85,	133	30.4	61,	144	17.8	36,
124	41.1	82,	134	28.3	56.5,	146	12.8	26,
125	39	78,	135	28	56,	147	9.9	20,
126	35.2	70.5,	136	28	56,	148	10	20,
127	37	74,	137	28	56,	150	7.4	15,
128	35	70,	138	24.5	49,	152b	6	12,

Day 152. Perfuser drained and refilled with 250 ml. of approximately 200 ppm. 2,4-DCP solution. Solution sampled before adding to the perfuser and after 1 hr. perfusion.

152ba	108	216,	156	33	66,	162	2.8	5.5,
152alhr	56.5	113,	158	23.6	47,	164	2.8	5.5,
154	30.7	61,	160	5.4	11,	165b	/	/,

Table 41d. continued.

Day 165. Perfuser drained and refilled with 250 ml. of approximately 200 ppm. 2,4-DCP solution. Solution sampled before adding to the perfuser and after 1 hr. perfusion.

A.	G.	H.	A.	G.	H.	A.	G.	H.
165ba	122	244,	167	37.3	75,	170	11.5	23,
165alhr	56	112,	168	23.5	47,	171b	3	6,
166	42.4	85,	169	22	44,			

Day 171. Perfuser drained and refilled as on Day 165.

171ba	88	176,	172	35	70,	174	11.6	23,
171alhr	64	128,	173	27	54,	175b	5.5	11,

Day 175. Perfuser drained and refilled as on Day 165.

175ba	100	200,	176	22	44,			
175alhr	69	138,	177b	11	22,			

Day 177. Perfuser drained and refilled as on Day 165.

177ba	98	196,	178	33	66,	180b	8	16,
177alhr	72.5	145,	179	24.6	49,			

Day 180. Perfuser drained and refilled as on Day 165.

180ba	100	200,	181	30.5	61,	182b	5.7	11,
180alhr	50.2	100,						

Day 182. Perfuser drained and refilled as on Day 165.

182ba	108	216,	183	37.8	76,	184b	18.4	37,
182alhr	68	136,						

Day 184. Perfuser drained and refilled as on Day 165.

184ba	105	210,	186	37	74,	189b	10	20,
184alhr	72	144,	187	33.2	66,			

Day 189. Perfuser drained and refilled as on Day 165.

189ba	99	198,	190	34.1	68,	192b	4.1	8,
189alhr	52	104,	191	18	36,			

Day 192. Perfuser drained and refilled as on Day 165.

192ba	103	206,	193	31	62,	195b	5.6	11,
192alhr	58	116,	194	17.3	35,			

Table 41d. continued.

Day 195. Perfuser drained and refilled with 250 ml. of approximately 200 ppm. 2,4-DCP solution. Solution sampled before adding to the perfuser and after 1 hr. perfusion.

A.	G.	H.	A.	G.	H.	A.	G.	H.
195ba	95	190,	196	28	56,	198b	3.4	7,
195alhr	55	110,	197	14.3	29,			

Day 198. Perfuser drained and refilled as on Day 195.

198ba	100	200,	199	24.3	49,			
198alhr	51.1	102,	200b	11.5	23,			

Day 200. Perfuser drained and refilled as on Day 195.

200ba	100	200,	202	30.2	60,	213b	1.6	3,
200alhr	.64	128,	203	13.6	27,			
201	44	88,	204	6.5	13,			

Day 213. Perfuser drained and refilled as on Day 195.

213ba	104	208,	215	30	60,	218	23.6	47,
213alhr	53	106,	216	30	60,	219	18	36,
214	33	66,	217	28.4	57,	220b	8	16,

Day 220. Perfuser drained and refilled as on Day 195.

220ba	95	190,	222	29.5	59,	225b	2.7	5,
220alhr	53.5	107,	223	21.5	43,			
221	34.2	68,	224	13	26,			

Day 225. Perfuser drained and refilled with 2,4-DCP as on D.195.

225ba	107	214,	227	32.6	65,	230b	8	16,
225alhr	66	132,	228	28	56,			
226	42.2	84,	229	15.8	32,			

Day 230. Perfuser drained and refilled as on Day 195.

230ba	101	202,	231	42.4	85,	233	25.3	51,
230alhr	68.4	137,	232	30.8	62,	234b	11.6	23,

Day 234. Perfuser drained and refilled as on Day 195.

234ba	92.5	185,	236	40	80,	239	4.8	10,
234alhr	59.5	119,	237	20.6	41,			
235	49.5	99,	238	8.8	18,			

Table 41e. Transferred adaptation to 2,4-D followed by 2,4-DCP.

Key to columns in table: as in Table 41, above.

Perfusion started on 16/8/51 with 50 gm of soil (2 to 4 mm., Sussex Lodge soil, dried June 1951,), and 250 ml. of 1,000 ppm. 2,4-D solution prepared from the"active"perfusate drained from a 2,4-D enriched perfuser (Graph and Table 42,) on 16/8/51. First sample taken after 1 hr. perfusion.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
0	67.4	86, 88, 94, 99,	22, 22, 24, 31,	4.5,4.5,	800
4	58.8	82, 85, 89,106,	27, 31, 31, 31,	5, 5,	700
6	58.7	82, 96,103,113,	24, 26, 34, 41,	5, 5,	700
8	70.0	73, 80, 91,107,	21, 26, 29, 30,	4, 9,	700
10	67.2	89, 91,101,104,	25, 27, 28, 30,	4.5,4.5,	750
12	72.8	92, 96,112,112,	58, 60, 62, 67,	7, 7,	350
14	62.6	106.107,120,125,	99,102,102,122,	95,100,	<10
Day 16. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.					
16a	56.4	92, 98,103,115,	30, 32, 36, 37,	5.5, 7,	600
20	51.6	99,113,117,117,	68, 74, 76,103,	25, 27,	90
22	51.8	106,106,116,145,	106,114,114,135,	110,132,	<10
Day 25. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.					
25a	45.4	86, 93, 95, 99,	35, 38, 38, 42,	4.5,6.5,	550
29	56.0	77, 81, 91,108,	27, 35, 38, 39,	5.5,5.5,	600
31	62.6	90,102,104,107,	43, 45, 56, 61,	6.5, 8,	300
Day 34. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.					
34a	66.5	66, 68, 69,101,	20, 24, 26, 27,	4.5,4.5,	1,000
38	57.5	85, 90,115,118,	52, 57, 61, 71,	5, 10,	250
40	48.5	79, 89,101,134,	83, 85,103,132,	68, 74,	15
42b	73.0	108,110,114,119,	75, 84, 93, 99,	100,103,	<10
Day 42. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.					
42a	73.0	74, 75, 78, 82,	19, 21, 21, 25,	4,5.5,	1,100
46	58.4	120,122,125,134,	134,138,140,140,	122,130,	<10
48	67.6	99,101,102,104,	59, 64, 73,107,	77,112,	<10
Day 49. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.					
49a	69.1	56, 65, 72, 72,	20, 23, 25, 26,	4.5,4.5,	1,000
53	72.3	73, 86, 97,110,	42, 44, 48, 55,	5.5,8.5,	400
55	72.4	90, 90,104,105,	94, 98,115,116,	90,112,	<10

Table 41e. continued.

Day 57. Perfuser drained and refilled with 250 ml. of approximately 200 ppm. 2,4-DCP solution. Solution sampled before adding to the perfuser and after 1 hr. perfusion.

A.	G.	H.	A.	G.	H.	A.	G.	H.
57ba	107	214,	58	40	80,	60	18	36,
57alhr	68	136,	59	27.1	54,	61b	6.2	12,

Day 61. Perfuser drained and refilled with 250 ml. of approximately 200 ppm. 2,4-DCP solution. Solution sampled before adding to the perfuser and after 1 hr. perfusion.

61ba	110	220,	73	42.5	85,	93	12.5	25,
61alhr	64	128,	75	38.4	77,	95	6	12,
62	57	114,	77	30	60,	97	4.3	9,
63	51	102,	79	32.7	65,	99	5	10,
64	51.5	103,	81	35.8	72,	101	8	16,
65	/	/,	83	32.8	66,	103	9	18,
67	46	92,	85	32	64,	105	7	14,
69	41.6	83,	87	23.7	47,	107	7.2	14,
70	38.9	78,	89	22.8	46,	109b	6.5	13,
71	43.9	88,	91	16	32,			

Day 109. Perfuser drained and refilled with 250 ml. of approximately 200 ppm. 2,4-DCP solution. Solution sampled before adding to the perfuser and after 1 hr. perfusion.

109ba	104	208,	113	38	76,	118	34.7	69,
109alhr	66	132,	114	45	90,	119	35.2	70,
110	55.5	111,	115	28.3	57,	120	36.9	74,
111	48	96,	116	41.5	83,			
112	44	88,	117	39.5	79,			

Table 42. Direct perfusion of 2,4-D, followed by 2,4-DCP and later by 2,4-D / 2,4-DCP mixture.

Key to columns in table:

A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. (relative to the 2,4-D component,) as % mean control.
D. Longest roots at nominal dilution concentration of 0.1 ppm. (relative to the 2,4-D component,) as % mean control.
E. Longest roots at nominal dilution concentration of 1.0 ppm. (relative to the 2,4-D component,) as % mean control.
F. Indicated 2,4-D concentration in the perfusate (ppm.).
G. Colourimeter reading (E.E.L. instrument,) in divisions.
H. Indicated 2,4-DCP concentration in the perfusate (ppm.).

Perfusion started on 31/5/51 with 60 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried October 1950,) and 250 ml. of 1,000 ppm. 2,4-D solution (common sterile bulk solution used for this perfuser and those of Tables 38a, 41a, and 41d,). Solution sampled before adding to the perfuser and after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0ba.	40.0	88,	95,	98,	105,	28,	30,	35,	55,	5,	5,	600
0alhr	40.0	85,	90,	105,	110,	40,	43,	43,	48,	5,	7.5,	450
8	39.8	78,	83,	83,	110,	43,	45,	48,	48,	5,	7.5,	500
32	43.7	105,	116,	121,	133,	94,	96,	96,	108,	99,	133,	<10

Day 36. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

36a	40.6	79,	81,	81,	84,	37,	39,	42,	59,	5,	5,	1,000
38	37.7	101,	104,	106,	117,	53,	53,	58,	69,	5.5,	8,	300
40	37.2	83,	89,	97,	116,	48,	51,	54,	59,	8,	11,	300
42	35.2	85,	101,	114,	116,	82,	91,	97,	106,	37,	43,	50
44	38.5	99,	114,	125,	135,	94,	94,	101,	122,	89,	99,	<10
46	32.8	85,	88,	88,	98,	101,	107,	122,	122,	119,	128,	<10

Day 49. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

49a	37.6	64,	67,	72,	85,	32,	35,	37,	43,	5.5,	8,	550
52	30.9	104,	107,	123,	159,	49,	49,	52,	68,	6.5,	6.5,	300
54	37.0	79,	79,	89,	116,	59,	62,	68,	76,	8,	8,	200
56	38.4	86,	89,	99,	110,	70,	76,	78,	78,	20,	24,	100
58	33.6	101,	101,	110,	134,	98,	107,	110,	134,	71,	104,	<10

Day 62. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

62a	42.8	84,	87,	87,	89,	37,	44,	47,	51,	7,	9.5,	400
64	43.6	53,	55,	67,	78,	39,	39,	44,	64,	7,	7,	500
66	40.1	100,	105,	107,	115,	55,	57,	62,	70,	5,	5,	200

Table 42. continued.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
68	43.5	83, 88, 88, 92,	53, 53, 53, 71,	4.5, 7,	300
70	64.2	104,106,107,124,	31, 33, 36, 37,	4.5, 6,	550
72	64.2	83, 90, 97,110,	42, 47, 72, 75,	9.5, 11,	250
74	45.2	122,131,144,146,	128,133,142,151,	91,151,	<10

Day 77. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 2 hr.

77a	67.4	70, 71, 80, 80,	19, 22, 27, 30,	3, 3,	1,000
80	64.1	92, 93, 97,100,	42, 44, 45, 47,	8, 8,	400
82	58.7	97, 99,107,128,	29, 31, 34, 39,	5, 7,	550
84	70.0	116,116,119,121,	34, 37, 39, 56,	4, 9,	500
86	77.7	77, 84, 84,102,	36, 40, 43, 50,	5, 5,	500
88	67.1	101,101,103,104,	69, 73, 75, 77,	12, 18,	130
90	74.8	79, 80,101,107,	91, 92,100,108,	67, 71,	15

Day 91. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding to the perfuser and after 1 hr.

A.	G.	H.	A.	G.	H.	A.	G.	H.
91ba.	90	180,	92b.	6.5	13,			
91a1hr.	70	140,	(this figure was checked,).					

Day 92. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding to the perfuser and after 2.5 hrs., 16 and 21 hrs.(Day 93,).

92ba.	98	196,	93a16hr.	35	70,
92a2.5hr.	54.5	109,	93a21hr.	19.2	38,

Day 93. Drained and refilled with 250 ml. of 200 ppm. 2,4-DCP.

93ba.	103	206,	100	39	78,	108	30	60,
93a1hr.	59	118,	101	38	76,	109	29.5	59,
94	51	102,	102	36	72,	110	27.1	54,
95	47	94,	103	36	72,	111	25	50,
96	43	86,	104	34.6	69,	112	23.2	46,
97	45	90,	105	35	70,	113b.	17.1	34,
98	35	70,	106	34.5	69,			
99	40	80,	107	32.5	65,			

Day 113. Drained and refilled with 250 ml. of 200 ppm. 2,4-DCP.

113ba.	115	230,	116	50	100,	120	44.5	89,
113a1hr.	67	134,	117	46.5	93,	121	42	84,
114	52	104,	118	50.5	101,	122b.	40.6	81,
115	52	104,	119	47	94,			

Table 42. continued.

Day 122. 25 ml. of 1% 2,4-D solution added to the perfuser without draining, leaving the 2,4-DCP concentration little changed and the 2,4-D concentration in the perfusate at 100 ppm. Sampled for 2,4-D and 2,4-DCP after 1 hr. perfusion.

A.	G.	H.	A.	G.	H.	A.	G.	H.
122a.	41	82,	132	19.5	39,	142	1.8	4,
123	35	70,	133	10	20,	144	2.0	4,
124	34.5	69,	134	5.6	11,	146	3.1	6,
125	29.4	59,	135	3.7	7,	147	3.4	7,
126	34.4	69,	136	4.7	9,	148	2.1	4,
127	28.5	57,	137	4.7	9,	149	/	/,
128	31	62,	138	2.7	5,	150	2.2	4,
129	28.6	57,	139	/	/,	152	1.6	3,
130	31.5	63,	140	2.2	4,	154	2.9	6,
131	20	40,	141	/	/,			

Day 156. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Sampled for 2,4-D and 2,4-DCP after 1 hr.

156a.	0.2	0,	163	0	0,	167	1.0	2,
158	0	0,	165	3.2	6,	169	3.0	6,
161	2	4,	166	/	/,	171	0.6	1,

Day 177. Perfuser drained and refilled with 250 ml. of solution containing 200 ppm. 2,4-D and 200 ppm. 2,4-DCP. Sampled for 2,4-DCP before adding to perfuser and 2,4-D and 2,4-DCP after 1 hr. perfusion.

177ba.	77.5	155,	179	29.9	60,	182b.	5.4	11,
177alhr.	64	128,	180	25.6	51,			
178	31	62,	181	10	20,			

Day 182. 25 ml. of perfusate removed and replaced by 25 ml. of 0.2% 2,4-DCP solution. Sampled after 1 hr.

182a.	45	90,	184	38	76,	186	20.7	41,
183	38.8	78,	185	29.3	59,	187b.	6.4	13,

Day 187. 25 ml. of 0.2% 2,4-DCP solution added. Sampled after 1 hr. perfusion.

187a.	46	92,	191	27.4	55,
188	42.5	85,	192	17.2	34,
189	37	74,	193b.	7.1	14,
190	29	58,			

Day 193. 10 ml. of 0.2 % 2,4-DCP solution. Sampled after 1 hr.

193a.	18.3	36,
194	16	32,
195b.	6	12,

Table 42. continued.

A.	G.	H.	A.	G.	H.	A.	G.	H.

Day 195. 10 ml. of 0.2% 2,4-DCP solution added to the perfuser without draining. Sampled after 1 hr. perfusion.

195a.	18.5	37,	199	4	8,	203	1.6	3,
196	12	24,	200	2.5	5,	204	0	0,
197	7.2	14,	201	2.1	4,			
198	2.7	5,	202	4.4	9,			

Day 122. Details of refill on previous page.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
122	72.3	61,	74,	80,	87,	18,	18,	22,	26,	4,	5.5,	1,200
124	54.9	80,	84,	93,	113,	20,	24,	24,	38,	3.5,	3.5,	900
126	69.1	51,	51,	52,	75,	20,	20,	22,	32,	6,	6,	1,150
128	72.9	67,	69,	70,	84,	19,	21,	22,	25,	3,	3,	1,050
130	72.3	60,	60,	65,	69,	23,	25,	25,	35,	4,	5.5,	900
132	72.4	51,	51,	53,	61,	25,	28,	30,	55,	5.5,	7,	700
134	67.4	59,	78,	86,	96,	24,	24,	24,	30,	4.5,	4.5,	900
136	67.7	65,	67,	75,	86,	22,	22,	22,	30,	6,	12,	1,000
138	57.4	68,	68,	89,	96,	24,	30,	31,	38,	5,	5,	750
140	56.5	55,	62,	71,	90,	19,	25,	30,	30,	7,	11,	700
142	43.4	74,	76,	78,	88,	21,	23,	28,	51,	7,	16,	700
144	44.2	84,	91,	91,	97,	32,	34,	41,	56,	4.5,	7,	650
146	43.2	81,	95,	100,	116,	32,	32,	37,	39,	7,	7,	500
148	43.1	100,	102,	121,	140,	26,	30,	30,	40,	7,	9.5,	550
150	49.8	80,	96,	96,	108,	32,	42,	46,	52,	8,	10,	400
152	64.0	80,	83,	91,	91,	67,	69,	80,	84,	20,	33,	90
154	60.7	106,	110,	110,	120,	74,	83,	89,	102,	64,	91,	10
156b	66.8	72,	76,	79,	87½	60,	61,	66,	117,	94,	100,	<10

Day 156. Details of refill as on previous page.

156a	63.8	61,	72,	107,	107,	27,	28,	28,	31,	3,	8,	750
159	66.4	56,	59,	60,	63,	20,	21,	23,	23,	3,	3,	1,000
161	64.4	84,	87,	90,	98,	23,	25,	25,	28,	4.5,	6,	750
163	68.3	79,	92,	103,	128,	26,	26,	26,	40,	4.5,	9,	800
165	67.0	69,	75,	79,	103,	37,	39,	57,	72,	6,	7.5,	450
167	74.7	83,	83,	101,	103,	59,	63,	67,	80,	9.5,	9.5,	300
169	75.2	67,	85,	88,	91,	67,	67,	71,	101,	68,	81,	10
171b	72.9	61,	80,	81,	88,	69,	72,	96,	100,	66,	100,	<10

Day 171. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

171a.	81.6	71,	76,	78,	97,	20,	20,	21,	28,	4,	4,	1,150
176	59.4	73,	74,	98,	121,	24,	29,	30,	49,	5,	5,	650

Day 177. Details of refill as on previous page.

Table 42. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
177a	55.9	57,	65,	66,	75,	9,	11,	12,	13,	2,	2,	200
179	52.7	44,	57,	70,	76,	9,	13,	15,	17,	2,	2,	200
180	68.3	40,	41,	53,	65,	7,	9,	12,	12,	1.5,	3,	210
182	66.0	36,	44,	45,	51,	9,	10,	12,	17,	1.5,	3,	220
184	67.4	52,	53,	56,	59,	7,	9,	9,	14,	1.5,	1.5,	250
186	60.0	45,	50,	53,	110,	15,	15,	17,	18,	0,	1.5,	170
187	67.6	33,	41,	47,	56,	9,	10,	13,	14,	1.5,	1.5,	200
188	70.7	31,	33,	50,	57,	11,	12,	13,	13,	3,	3,	220
190	76.3	45,	45,	46,	59,	10,	12,	17,	18,	2.5,	2.5,	200
193	75.4	39,	61,	68,	79,	10,	13,	15,	16,	2.5,	2.5,	210
196	71.4	49,	50,	53,	59,	11,	14,	16,	18,	3,	3,	180
199	60.3	73,	80,	83,	88,	11,	13,	17,	18,	3.5,	5,	180
213	73.3	31,	48,	52,	59,	9,	12,	15,	17,	1.5,	1.5,	210
216	81.3	31,	33,	56,	61,	15,	15,	16,	16,	2.5,	3.5,	170
219	77.7	68,	77,	81,	86,	59,	62,	66,	76,	59,	67,	2
222b	72.0	96,	96,	99,	106,	107,	109,	120,	127,	79,	96,	<1
Day 222. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.												
222a	72.0	45,	52,	57,	59,	14,	14,	15,	18,	3,	3,	200
225	60.8	84,	90,	100,	107,	84,	87,	89,	102,	18,	26,	10
228b	59.7	87,	101,	102,	116,	101,	114,	119,	125,	44,	70,	3
Day 228. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.												
228a	59.7	64,	72,	74,	82,	23,	25,	27,	30,	4,	5.5,	90
230	59.1	100,	108,	113,	127,	39,	39,	41,	49,	6,	12,	50
232	66.3	83,	98,	102,	108,	69,	96,	96,	102,	36,	44,	5
234	51.2	104,	104,	106,	111,	107,	111,	117,	136,	94,	125,	<1

Table 42a. Transferred adaptation to 2,4-D, followed by 2,4-D / 2,4-DCP mixture.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. (relative to the 2,4-D component,) as % mean control.
D. Longest roots at nominal dilution concentration of 0.1 ppm. (relative to the 2,4-D component,) as % mean control.
E. Longest roots at nominal dilution concentration of 1.0 ppm. (relative to the 2,4-D component,) as % mean control.
F. Indicated 2,4-D concentration in the perfusate (ppm.).
G. Colourimeter reading (E.E.L. instrument,) in divisions.
H. Indicated 2,4-DCP concentration in the perfusate (ppm.).

Perfusion started on 16/8/52 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried February 1952,) and 250 ml. of 100 ppm. 2,4-D solution prepared from the "active" perfusate drained from a 2,4-D enriched perfuser (Table 42f,) on 13/8/52. First sample after 1 hr.perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	61.9	39,	42,	53,	53,	16,	18,	18,	20,	3,	5,	130
3	65.8	47,	53,	62,	62,	15,	17,	21,	24,	3,	3,	125
6	49.6	89,	107,	107,	139,	95,	97,	99,	107,	73,	73,	1.5
9b	49.7	99,	103,	115,	117,	99,	101,	103,	105,	59,	63,	2
Day 9. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.												
9a	62.8	69,	75,	76,	78,	19,	19,	22,	22,	3,	5,	120
11	49.7	81,	87,	101,	105,	36,	44,	46,	46,	8,	10,	35
13	86.2	76,	80,	84,	120,	104,	113,	113,	116,	79,	84,	1
15b	68.8	90,	93,	99,	106,	87,	89,	90,	96,	64,	73,	1.5
Day 15. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.												
15a	76.6	59,	69,	69,	76,	14,	17,	17,	21,	2.5,	4,	145
17	71.9	50,	50,	52,	58,	28,	50,	50,	53,	11,	16,	30
20b	63.0	89,	89,	106,	106,	91,	92,	95,	97,	103,	106,	<1
Day 120. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.												
20a	63.0	60,	64,	67,	73,	17,	24,	24,	29,	3,	6.5,	95
22	66.9	81,	82,	82,	84,	87,	88,	99,	102,	66,	100,	<1
25b	61.2	91,	96,	98,	103,	88,	101,	104,	109,	52,	87,	1

Day 25. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 200 ppm. 2,4-DCP. Sampled for 2,4-DCP before adding, and for 2,4-D and 2,4-DCP after 1 hr.

Table 42a. continued.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
25a	61.2	51, 52, 56, 57,	18, 18, 19, 20,	3.5, 5,	125
28	40.1	72, 75, 80, 82,	25, 30, 30, 30,	5, 5,	90
31	61.3	41, 51, 52, 54,	19, 20, 21, 26,	3.5,3.5,	100
34	52.8	49, 53, 61, 74,	19, 19, 21, 21,	4,5.5,	115
37	59.8	39, 45, 49, 57,	15, 17, 17, 23,	3.5, 5,	135
40	61.6	49, 55, 57, 73,	19, 20, 21, 28,	3, 5,	95
46	56.7	55, 55, 58, 79,	16, 16, 23, 23,	3.5,5.5,	125
49	63.5	50, 52, 63, 75,	19, 19, 19, 20,	1.5, 3,	120
52	74.3	62, 63, 65, 88,	26, 31, 32, 34,	5.5, 8,	70
55	77.6	80, 85, 90, 97,	81, 84, 88, 94,	77, 81,	1
58	64.8	88, 93, 99,119,	74, 86, 91,103,	86, 96,	<1
61b	76.5	113,117,123,123,	85, 90, 90,109,	92, 97,	<1
Day 61. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.					
61a	76.5	68, 81, 84, 88,	26, 27, 30, 30,	4, 4,	80
64	76.3	85,100,108,114,	76, 87,105,110,	87,102,	<1
66b	75.0	100,115,117,121,	100,108,115,117,	84, 92,	<1
Day 66. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.					
66a	75.0	95, 96,100,111,	20, 21, 25, 27,	4,5.5,	95
68	75.0	87, 89, 96, 99,	83, 88, 93,111,	97,117,	<1
70b	65.4	101,104,116,116,	92, 92,107,110,	75, 78,	1
Day 70. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 200 ppm. 2,4-DCP. Sampled for 2,4-DCP before adding, and for 2,4-D and 2,4-DCP after 1 hr.					
70a	65.4	61, 63, 69, 72,	18, 20, 21, 26,	3,4.5	110
73	65.4	63, 66, 73, 78,	23, 23, 28, 28,	3,4.5,	100
76	68.6	66, 67, 77, 90,	19, 20, 25, 29,	3,4.5,	105
79	65.2	61, 63, 74,104,	20, 22, 23, 23,	4.5, 6,	90
82	50.0	78, 80, 82, 84,	26, 34, 36, 42,	6, 10,	60
85	65.0	74, 75, 80, 97,	48, 52, 57, 60,	14, 17,	30
88	64.7	97,100,102,114,	102,103,108,113,	91,111,	<1
91b	74.4	83, 85, 86, 96,	69, 82, 90, 90,	96,106,	<1
Day 91. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.					
91a	59.7	57, 59, 60, 67,	22, 23, 27, 28,	3.5, 5,	90
94	75.1	95, 96, 97,111,	90,100,105,108,	93, 96,	<1
96	72.5	88, 88,108,110,	97,102,103,106,	74, 79,	1
Day 96. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 200 ppm. 2,4-DCP. Sampled for 2,4-DCP before adding, and 2,4-D and 2,4-DCP after 1 hr.					

Table 42a. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
96a	72.5	40,	43,	51,	51,	16,	17,	18,	21,	3,	4,	130
98	76.2	76,	91,	97,	101,	28,	30,	32,	37,	4,	5.5,	70
100	70.5	89,	89,	92,	95,	33,	35,	41,	50,	4.5,	5.5,	55
102	83.0	72,	79,	84,	90,	27,	30,	35,	36,	2.5,	3.5,	65
104	63.7	74,	80,	86,	97,	66,	67,	67,	71,	22,	27,	10
106	64.0	94,	97,	105,	122,	55,	58,	66,	77,	25,	27,	8
108	58.3	81,	86,	96,	98,	55,	57,	62,	72,	74,	77,	1
110	45.1	82,	82,	109,	124,	84,	86,	100,	102,	91,	106,	<1

Day 112. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

112a	62.9	73,	78,	86,	92,	12,	13,	19,	19,	5,	5,	120
114	81.6	82,	83,	88,	103,	27,	27,	31,	32,	6,	6	75
116	91.0	91,	92,	92,	93,	86,	92,	92,	103,	88,	93,	<1
118	85.1	93,	94,	95,	95,	88,	89,	96,	102,	90,	106,	<1

2,4-DCP results:

A.	G.	H.	A.	G.	H.	A.	G.	H.
25ba.	90	180,	37	33	66,	50	9.4	19,
25alhr.	62	124,	38	30.3	61,	51	8	16,
26	49.5	99,	39	31	62,	52	7.6	15,
27	49	98,	40	26	52,	53	7.3	15,
28	44	88,	41	25.4	51,	54	6.4	13,
29	43.5	87,	42	25	50,	55	6.8	14,
30	41.1	82,	43	27.1	54,	56	5.4	11,
31	40.9	82,	44	24	48,	57	5.2	10,
32	37.7	75,	45	23.5	47,	58	5.4	11,
33	35.7	71,	46	19.4	39,	59	4.3	9,
34	34.5	69,	47	19	38,	60	3.8	8,
35	37.0	74,	48	15.1	30,	61b	6.1	12,
36	31.7	63,	49	11.6	23,			

Day 61. drained and refilled with 250 ml. of 100 ppm. 2,4-D.

61alhr.	1.2	2,	65	2.7	5,	69	1.3	3,
62	2.6	5,	66	3.1	6,	70b.	2.3	5,
63	2.6	5,	67	1.3	3,			
64	4.1	8,	68	0.7	1,			

Day 70. Drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 200 ppm. 2,4-DCP.

70ba.	100	200,	72	46.5	93,	75	36.4	73,
70alhr.	68.5	137,	73	41.0	82,	76	35	70,
71	51.5	103,	74	38	76,	77	32	64,

Table 42a. continued.

A.	G.	H.	A.	G.	H.	A.	G.	H.
78	32.1	64,	83	8.3	17,	88	6.2	12,
79	28.4	57,	84	7.6	15,	89	5.7	11,
80	23.6	47,	85	8.1	16,	90	2.9	6,
81	18.8	38,	86	7	14,	91	5	10,
82	13	26,	87	6.2	12,	92	2.3	5,

Day 96. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 100 ppm. 2,4-DCP.

96ba.	100	200,	103	23	46,	111	4.8	10,
96alhr.	68	136,	104	12.4	25,	112	5.1	10,
97	54	108,	105	9.1	18,	113	0	0,
98	48	96,	106	9.8	20,	114	0	0,
99	43.2	86,	107	7.6	15,	115	0	0,
100	38	76,	108	6.2	12,	116	0	0,
101	33.7	67,	109	6.2	12,	117	0	0,
102	28.3	57,	110	4.6	9,	118b.	0	0,

Day 118. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

118ba.	52.5	105,	119	19	38,	121b.	5.1	10,
118alhr.	32.5	65,	120	5.4	11,			

Day 121. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

121ba.	44	88,	122	8.9	18,
121alhr.	25.6	51,	123	3.1	6,

Table 42b. Direct perfusion of 2,4-D followed by mixture containing 2,4-D and 2,4-DCP.

Key to columns in table:

A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. (relative to the 2,4-D component,) as % mean control.
D. Longest roots at nominal dilution concentration of 0.1 ppm. (relative to the 2,4-D component,) as % mean control.
E. Longest roots at nominal dilution concentration of 1.0 ppm. (relative to the 2,4-D component,) as % mean control.
F. Indicated 2,4-D concentration in the perfusate (ppm.).
G. Colourimeter reading (Unicam instrument,) in divisions.
H. Indicated 2,4-DCP concentration in the perfusate (ppm.).

Perfusion started on 8/2/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 100 ppm. 2,4-D solution. Solution sampled before adding to perfuser.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	84.2	56,	57,	69,	81,	15,	18,	20,	21,	3.5,	7,	125
5	76.3	33,	35,	37,	38,	13,	13,	14,	15,	2.5,	4,	110
10	82.6	53,	56,	66,	97,	12,	12,	15,	17,	3.5,	3.5,	130
15	84.1	48,	56,	59,	66,	14,	15,	16,	17,	5,	6,	65
21	82.0	73,	74,	77,	79,	85,	86,	88,	89,	43,	54,	3
24b	86.1	91,	94,	101,	118,	91,	93,	96,	103,	79,	91,	1

Day 24. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

24a	83.5	54,	62,	63,	93,	13,	13,	14,	15,	3.5,	3.5,	130
26	75.9	70,	74,	76,	79,	21,	24,	25,	30,	4,	4,	85
28	76.0	43,	58,	62,	78,	18,	20,	22,	24,	6.5,	9,	45
30	91.3	73,	75,	82,	83,	91,	98,	102,	105,	62,	84,	1.5
32	88.4	82,	84,	87,	88,	73,	80,	84,	86,	77,	79,	1
34b	84.1	74,	87,	88,	88,	81,	81,	82,	97,	57,	85,	1.5

Day 34. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

34a	78.7	34,	41,	41,	70,	12,	14,	17,	18,	5,	6.5,	140
36	71.0	83,	93,	96,	103,	39,	39,	41,	52,	10,	10,	50
38	82.7	105,	105,	113,	118,	99,	100,	105,	114,	95,	110,	1
40b	71.1	93,	94,	100,	105,	70,	72,	76,	78,	88,	88,	1

Day 40. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

40a	72.5	66,	67,	70,	71,	16,	18,	18,	18,	2.5,	4,	130
42	73.3	83,	85,	87,	96,	60,	69,	71,	72,	13,	17,	15
44b	76.7	108,	114,	116,	118,	112,	113,	116,	117,	110,	110,	1

Day 44. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

Table 42b. continued.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
44a	76.7	75, 79, 82, 91,	19, 21, 23, 30,	5,6.5,	100
46	79.9	103,103,105,115,	103,111,121,129,	86,129,	<1
48b	84.6	74, 87,100,104,	88, 91, 95,103,	88, 91,	<1

Day 48. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

48a	77.1	88, 88, 88, 94,	18, 19, 21, 23,	4, 8,	100
50b	77.1	96,101,102,113,	104,107,111,126,	93,102,	<1

Day 50. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 200 ppm. 2,4-DCP. Solution sampled for 2,4-DCP before adding to the perfuser and for 2,4-D and 2,4-DCP after 1 hr. perfusion.

50a	77.1	84, 84, 94, 94,	15, 18, 20, 21,	2.5,3.5,	125
52	82.5	57, 58, 68, 68,	15, 16, 17, 25,	5, 5,	160
53	85.2	48, 55, 60, 63,	15, 16, 16, 17,	3.5,3.5,	160
56	74.7	63, 64, 65, 68,	17, 18, 18, 19,	4,5.5,	140
59	81.9	62, 63, 65, 76,	18, 21, 23, 26,	2.5,3.5,	120
62	76.0	70, 75, 78, 84,	24, 30, 34, 37,	5,6.5,	70
65	80.0	69, 69, 71, 79,	17, 19, 23, 25,	4, 5,	110
68	81.7	82, 84, 94, 98,	22, 28, 32, 37,	5, 6,	70
71	73.9	84, 92, 95, 99,	24, 24, 26, 28,	8,9.5,	55
74	76.7	92, 96, 96,105,	100,108,109,116,	92,116,	<1
77b	82.0	96, 99,114,114,	107,109,110,110,	96,106,	<1

Day 77. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 200 ppm. 2,4-DCP. Solution sampled for 2,4-DCP before adding to the perfuser and for 2,4-D and 2,4-DCP after 1 hr. perfusion.

77a	69.9	71, 80, 81, 81,	21, 21, 23, 23,	5.5, 7,	105
80	81.9	40, 42, 44, 52,	12, 13, 16, 17,	2.5,2.5,	170
83	75.5	71, 76, 82, 84,	21, 24, 25, 32,	2.5, 4,	90
86	83.9	70, 74, 79, 86,	14, 15, 16, 17,	2.5,3.5,	160
89	80.6	74, 77, 78, 87,	17, 18, 21, 24,	2.5,3.5,	140
92	88.6	77, 80, 87, 87,	21, 23, 24, 27,	3.5,3.5,	95,
95	75.1	85, 86, 90,101,	17, 18, 19, 21,	2.5,2.5,	110
98	80.3	97, 98,105,124,	26, 27, 30, 31,	8.5, 11,	50
101	90.0	101,106,108,108,	94, 94, 96,102,	101,103,	<1

Day 104. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 200 ppm. 2,4-DCP. Solution sampled for 2,4-DCP before adding to the perfuser and for 2,4-D and 2,4-DCP after 1 hr. perfusion.

104a	88.3	68, 70, 73, 74,	17, 17, 17, 18,	3.5,4.5,	145
107	92.6	68, 73, 75, 91,	18, 19, 20, 22,	2, 3,	135
110	93.3	93, 93, 95,103,	24, 26, 26, 28,	7.5,7.5,	65
113	93.9	97, 98, 98,100,	103,104,105,112,	90, 93,	<1
116	92.3	98,100,102,102,	98,100,103,105,	102,104,	<1

Table 42b. continued.

2,4-DCP concentrations in the perfusate during the periods of perfusion with 2,4-D/2,4-DCP mixture.

A.	G.	H.	A.	G.	H.	A.	G.	H.
50ba	7.0	232,	59	46.0	51,	69	71.5	22,
50alhr	15.0	123,	60	45.0	52,	70	74.4	19,
51	24.0	93,	61	45.0	52,	71	79.4	15,
52	24.5	92,	62	51.7	43,	72	84.3	11,
53	29.5	79,	63	54.6	39,	73	88.7	8,
54	34.1	70,	64	55.5	38,	74	86.8	9,
55	35.0	68,	65	58.0	36,	75	87.7	9,
56	38.5	62,	66	58.0	36,	76	89.8	7,
57	42.8	55,	67	64.2	29,	77b	94.4	5,
58	47.1	49,	68	69.4	24,			

Day 77. Refilled with 2,4-D/2,4-DCP mixture.

77ba	9.7	195,	86	31.1	76,	96	84.4	11,
77alhr	12.9	133,	87	31.1	76,	97	83.5	12,
78	19.8	106,	88	32.9	72,	98	85.1	11,
79	20.9	102,	89	33.4	71,	99	85.7	10,
80	22.0	99,	90	38.3	62,	100	87	9,
81	24.4	92,	91	42.8	55,	101	86.1	10,
82	25.2	90,	92	51.5	43,	102	86.2	10,
83	26.3	87,	93	72.2	21,	103	87.3	9,
84	27.8	83,	94	80.0	15,	104b	87	9,
85	29.5	79,	95	82.8	12,			

Day 104. Refilled with 2,4-D/2,4-DCP mixture.

104ba	6.8	251,	107	78	16,	111	88.4	8,
104alhr	10.8	155,	108	84.3	11,	112	89.0	8,
105	18.8	109,	109	87.0	9,	113	90.0	7,
106	30.4	78,	110	88.1	8,			

Table 42c. Transferred adaptation to 2,4-D, followed by 2,4-D / 2,4-DCP mixture.

Key to columns in table: as in Table 42, above.

Perfusion started on 28/7/52 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried February 1952,) and 250 ml. of 100 ppm. 2,4-D solution, prepared from the "active" perfusate drained from a 2,4-D enriched perfuser (Table 42f,) on 28/7/52. First sample taken after 1 hr.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
0	61.9	57, 58, 60, 79,	16, 18, 19, 20,	3, 5,	120
10	59.4	66, 77, 82,104,	98,101,108,125,	86, 98,	<1

Day 12. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

12a	51.0	82, 98,114,116,	26, 26, 29, 33,	6, 6,	80
14	67.5	71, 86, 93, 99,	25, 37, 37, 44,	6, 6,	55
16	55.4	92, 92, 92, 96,	63, 65, 74, 74,	12, 16,	20
18	55.7	104,115,122,128,	110,119,122,140,	97, 99,	<1

Day 20. 2.5 ml. of 1% 2,4-D solution added without draining, to make the perfusate approximately 250 ml. of 100 ppm. Sampled after 1 hr.

20a	58.6	43, 48, 50, 51,	22, 22, 22, 26,	5, 5,	100
22	65.8	53, 55, 56, 71,	24, 24, 29, 32,	4.5, 6,	70
24	53.0	87,102,106,127,	49, 51, 53, 57,	13, 17,	20,
26	61.9	94,103,108,138,	99,100,103,121,	74, 87,	1
28b	59.3	98, 98,112,113,	74, 95,106,113,	93,110,	<1

Day 28. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

28a	62.8	51, 61, 62, 67,	16, 19, 19, 29,	3, 5,	120
30	49.7	57, 61, 61, 63,	26, 28, 28, 38,	4, 6,	75
32	86.2	92, 94, 95, 99,	94, 99, 99,100,	104,109,	<1
34b	76.6	92, 95,100,112,	90, 98,100,114,	106,114,	<1

Day 34. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

34a	76.6	55, 61, 64, 67,	17, 17, 20, 22,	4, 4,	135
36	71.9	47, 51, 54, 61,	39, 39, 50, 65,	15, 18,	15
39b	66.9	91, 94, 94,105,	84, 88, 91,108,	64, 93,	1

Day 39. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 100 ppm. 2,4-DCP. Sampled for 2,4-DCP before adding to perfuser and 2,4-D and 2,4-DCP after 1 hr. perfusion.

39a	63.0	43, 49, 51, 56,	21, 22, 22, 24,	3,4.5,	100
42	66.9	60, 64, 88, 99,	19, 20, 22, 22,	3,4.5,	110
45	61.2	44, 52, 65, 70,	33, 33, 39, 41,	8, 17,	45

Table 42c. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
48	40.1	97,	110,	110,	110,	70,	72,	75,	85,	52,	60,	2.5
50b	61.3	98,	98,	100,	108,	87,	90,	107,	110,	90,	98,	1

Day 50. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 100 ppm. 2,4-DCP. Sampled for 2,4-DCP before adding to perfuser and 2,4-D and 2,4-DCP after 1 hr. perfusion.

50a	52.0	67,	73,	85,	88,	21,	25,	25,	27,	4,	6,	90
53	52.8	61,	74,	79,	91,	21,	25,	27,	30,	4,	5.5,	80
56	59.8	62,	64,	71,	79,	30,	32,	32,	32,	4,	5.5,	65
61	49.0	86,	90,	94,	106,	55,	57,	59,	77,	14,	17,	25
64	51.2	106,	106,	117,	123,	94,	100,	111,	115,	100,	102,	<1
66b	55.1	98,	100,	107,	109,	80,	82,	84,	85,	102,	124,	<1

Day 66. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

66a	55.1	62,	65,	71,	73,	27,	27,	31,	35,	3.5,	7.5,	80
68	63.5	76,	82,	91,	95,	69,	72,	72,	77,	95,	106,	<1
70	55.1	96,	102,	105,	107,	74,	80,	89,	89,	91,	94,	<1

Day 72. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

72a	77.5	53,	55,	58,	59,	18,	18,	18,	31,	4,	5,	100
74	77.6	81,	93,	95,	103,	66,	67,	82,	90,	80,	92,	1
76	61.6	91,	96,	98,	117,	98,	99,	99,	107,	46,	50,	3
78b	69.6	105,	106,	112,	118,	103,	105,	108,	108,	106,	111,	<1

Day 78. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 100 ppm. 2,4-DCP. Sampled for 2,4-DCP before adding to perfuser and 2,4-D and 2,4-DCP after 1 hr. perfusion.

78a	75.3	47,	49,	52,	55,	16,	17,	17,	18,	2.5,	4,	140
80	75.3	51,	52,	52,	61,	24,	24,	25,	29,	2.5,	4,	90
82b	76.5	73,	73,	81,	93,	33,	37,	38,	59,	11,	12,	40

Day 82. 12.5 ml. of 0.2% 2,4-DCP solution added without draining the perfuser, making the 2,4-DCP concentration of the perfusate approximately 100 ppm. Sampled after 1 hr. perfusion.

82a	76.5	69,	73,	79,	79,	34,	37,	39,	42,	9,	13,	50
84b	81.3	87,	90,	95,	103,	87,	89,	91,	94,	95,	102,	<1

Day 84. 12.5 ml. of 0.2% 2,4-DCP solution added without draining the perfuser, making the 2,4-DCP concentration of the perfusate approximately 100 ppm. Sampled after 1 hr. perfusion, for 2,4-DCP

86	80.3	90,	92,	97,	117,	81,	85,	92,	107,	76,	79,	1

Day 42c. continued.

A.	G.	H.	A.	G.	H.	A.	G.	H.
Day 39. Drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 100 ppm. 2,4-DCP.								
39ba.	56	112,	41	23.7	47,	44b.	4.8	10,
39alhr.	33.7	67,	42	19	38,			
40	28.8	58,	43	11.7	23,			
Day 44. 12.5 ml. of 0.2% 2,4-DCP solution added without draining.								
44alhr.	38	76,	47	13.3	27,	50b.	8.9	18,
45	26	52,	48	11.2	22,			
46	18	36,	49	9.1	18,			
Day 50. Drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 100 ppm. 2,4-DCP.								
50ba.	58	116,	51	27.1	54,	53	9.8	20,
50alhr.	40	80,	52	18.2	36,	54b.	7.5	15,
Day 54. 15 ml. of 0.2% 2,4-DCP solution added without draining.								
54alhr.	49.3	99,	56	17.9	36,	58b.	9	18,
55	31.3	63,	57	10	20,			
Day 58. 15 ml. of 0.2% 2,4-DCP solution added without draining.								
58alhr.	45	90,	59	26	52,	60b.	11.1	22,
Day 60. 15 ml. of 0.2% 2,4-DCP solution added without draining.								
60alhr.	46	92,	63	3.8	8,	66b.	3.5	7,
61	23	46,	64	4.4	9,			
62	4.5	9,	65	1.9	4,			
Day 66. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-DCP solution.								
Day 78. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 100 ppm. 2,4-DCP.								
78ba.	53.4	107,	79	27	54,	81	16.2	32,
78alhr.	37	74,	80	22.7	45,	82b.	4.5	9,
Day 82. 12.5 ml. of 0.2% 2,4-DCP solution added without draining.								
82alhr.	37.6	75,	83	18.9	38,	84b.	3.1	6,
Day 84. 12.5 ml. of 0.2% 2,4-DCP solution added without draining.								
84alhr.	36	72,	85	4.2	8,	86b.	2.0	4,
Day 86. 12.5 ml. of 0.2% 2,4-DCP solution added without draining.								
86alhr.	35.8	72,	87	2.2	4,			

Table 42d. Transferred adaptation to 2,4-D, followed by 2,4-D / 2,4-DCP mixture.

Key to columns in table: as in Table 42, above.

Perfusion started on 7/8/52 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried February 1952,), and 250 ml. of 100 ppm. 2,4-D solution, prepared from the "active" perfusate drained from a 2,4-D enriched perfuser (Table 42f,) on 7/8/52. First sample taken after 1 hr. perfusion.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
0	59.4	66, 67, 74, 74,	20, 24, 25, 30,	5,6.5,	90
3	50.9	67, 80, 84, 90,	20, 20, 23, 27,	1.5,3.5,	95
6	55.4	123,123,126,126,	76, 81,101,117,	96, 98,	<1
9b	61.9	76, 81, 81,100,	68, 68, 76, 76,	82,104,	<1

Day 9. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

9a	61.9	53, 60, 60, 70,	19, 20, 21, 23,	5, 5,	115
11	53.9	78, 80,102,124,	33, 37, 39, 58,	11, 11,	40
13	67.3	68, 82, 86,100,	68, 71, 79, 95,	55, 83,	2
15b	49.6	77, 79, 89,105,	93, 93, 95,109,	103,103,	<1

Day 15. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

15a	49.6	53, 53, 73, 81,	22, 24, 26, 30,	4, 6,	90
17	57.1	91, 95, 96,114,	98,107,110,114,	33, 39,	6
19b	49.7	105,111,125,125,	87, 97, 99,115,	101,111,	<1

Day 19. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

19a	49.7	65, 67, 79, 95,	20, 20, 22, 24,	4, 4,	105
21	43.4	88, 90, 90,106,	108,111,115,132,	102,108,	<1
23b	60.0	105,108,110,117,	108,112,113,120,	103,107,	<1

Day 23. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 100 ppm. 2,4-DCP. Solution sampled for 2,4-D before adding to the perfuser and for 2,4-D and 2,4-DCP after 1 hr. perfusion.

23a	60.0	63, 63, 63, 83,	20, 22, 22, 28,	3.5, 5,	100
25	73.6	71, 78, 80, 91,	33, 33, 34, 35,	9.5, 12,	45
27	72.0	92, 94, 96,124,	103,111,111,114,	85, 93,	<1
29	66.9	78, 87, 96, 99,	90, 94, 99,114,	81, 87,	<1

Day 29. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 100 ppm. 2,4-DCP. Solution sampled for 2,4-DCP before adding to the perfuser and for 2,4-D and 2,4-DCP after 1 hr. perfusion.

Table 42d. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
29a	63.0	43,	43,	46,	52,	16,	17,	19,	25,	3,	3,	140
31	59.6	60,	65,	72,	72,	13,	15,	17,	19,	3.5,	3.5,	140
33	71.8	64,	65,	70,	79,	18,	22,	22,	25,	4,	7,	100
35	61.2	67,	72,	87,	90,	26,	26,	29,	33,	10,	10,	80
37	51.8	83,	83,	85,	110,	35,	37,	39,	44,	6,	9.5,	45
39	49.2	96,	96,	116,	116,	39,	41,	43,	55,	12,	17,	30
41	52.0	92,	92,	96,	115,	89,	90,	92,	94,	54,	73,	2
43b	52.8	74,	76,	78,	82,	114,	116,	127,	131,	63,	91,	1.5
Day 43. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.												
43a	52.8	63,	64,	64,	71,	23,	27,	27,	30,	4,	4,	80
45	53.3	96,	96,	102,	105,	30,	36,	40,	41,	11,	13,	45
51	49.0	102,	106,	106,	118,	100,	104,	120,	122,	102,	118,	<1
53	51.2	102,	115,	121,	123,	107,	113,	123,	127,	88,	96,	<1
55.	56.7	81,	83,	93,	102,	92,	95,	97,	104,	74,	76,	1.5
57b	55.1	78,	78,	80,	89,	71,	71,	74,	94,	58,	85,	1.5
Day 57. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.												
57a	55.1	54,	56,	80,	87,	20,	20,	22,	29,	3.5,	5.5,	105
59	63.5	68,	69,	87,	90,	41,	48,	49,	58,	6.5,	9.5,	30
61	55.1	85,	85,	98,	103,	92,	96,	103,	123,	80,	85,	<1
Day 63. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.												
63a	77.5	66,	71,	79,	85,	23,	25,	25,	31,	4,	5,	90
65	77.6	77,	81,	85,	94,	89,	94,	97,	98,	66,	77,	1.5
67	61.6	96,	98,	101,	106,	63,	65,	86,	88,	75,	123,	<1
69b	69.6	115,	119,	122,	127,	80,	89,	91,	102,	105,	116,	<1
Day 69. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 100 ppm. 2,4-DCP. Solution sampled for 2,4-DCP before adding to the perfuser and for 2,4-D and 2,4-DCP after 1 hr. perfusion.												
69a	75.3	50,	54,	59,	59,	22,	25,	27,	33,	2.5,	4,	85
71	75.3	60,	73,	77,	88,	21,	23,	25,	27,	4,	4,	90
73b	76.5	80,	81,	85,	89,	29,	34,	35,	36,	6.5,	8,	55
Day 73. 12.5 ml. of 2,000 ppm. 2,4-DCP solution added without draining, making the perfusate approximately 100 ppm. 2,4-DCP. Sampled for 2,4-D and 2,4-DCP after 1 hr. perfusion.												
73a	76.5	86,	89,	92,	107,	27,	31,	33,	34,	6.5,	8,	60
75	81.3	81,	89,	89,	96,	71,	74,	78,	86,	33,	33,	10

Table 42d. continued.

Day 75. 12.5 ml. of 2,000 ppm. 2,4-DCP solution added without draining, making the perfusate approximately 100 ppm. 2,4-DCP. Sampled for 2,4-D and 2,4-DCP after 1 hr. perfusion.

75a		Assay sample rejected.	
77	80.3	96, 96, 97,102, 90, 92, 95, 97, 81,106,	<1

Day 77. 12.5 ml. of 2,000 ppm. 2,4-DCP solution added without draining, making the perfusate approximately 100 ppm. 2,4-DCP. Sampled for 2,4-DCP only after 1 hr.

79	76.4	85, 98, 98,109, 84, 90, 94,107, 71, 72,	1.5

2,4-DCP concentrations during the periods of perfusion with 2,4-D / 2,4-DCP mixtures.

Day 23. Perfuser drained and refilled with 2,4-D/2,4-DCP mixture.

A.	G.	H.	A.	G.	H.	A.	G.	H.
23ba	56.4	113,	23alhr	34.2	68,	24	18.7	37,
						25b	6.2	12,

Day 25. Approximately 13 ml. of 2,000 ppm. 2,4-DCP solution added without draining. Sampled for 2,4-DCP after 1 hr.

25alhr	37.1	74,	26	19.1	38,	27b	9.5	19,

Day 27. Approximately 15 ml. of 2,000 ppm. 2,4-DCP solution added without draining. Sampled for 2,4-DCP after 1 hr.

27alhr	45.0	90,	28	28.0	56,	29b	15.3	31,

Day 29. Perfuser drained and refilled with 2,4-D/2,4-DCP mixture.

29ba	56	112,	31	17	34,	34b	3.6	7,
29alhr	38.6	77,	32	9.6	19,			
3 0	29	58,	33	9.5	19,			

Day 34. Approximately 13 ml. of 2,000 ppm. 2,4-DCP solution added without draining. Sampled for 2,4-DCP after 1 hr.

34alhr	40.0	80,	38	10.2	20,	41	7	14,
35	28.3	57,	39	9.3	19,	42	6.7	13,
36	18.2	36,	40	8.5	17,	43	5.2	10,
37	11.4	23,						

Day 69. Perfuser drained and refilled with 2,4-D/2,4-DCP mixture.

69ba	53.4	107,	70	26	52,	72	16.9	34,
69alhr	39.3	79,	71	22.1	44,	73b	8.7	17,

Table 42d. continued.

Day 73. 12.5 ml. of 2,000 ppm. 2,4-DCP solution added without draining. Sampled for 2,4-DCP after 1 hr.

A.	G.	H.	A.	G.	H.	A.	G.	H.
73alhr	41.0	82,	74	21.0	42,	75b	8.3	17,

Day 75. 12.5 ml. of 2,000 ppm. 2,4-DCP solution added without draining. Sampled after 1 hr. for 2,4-DCP.

75alhr	41.5	83,	76	13.5	27,	77b	4.2	8,

Day 77. 12.5 ml. of 2,000 ppm. 2,4-DCP solution added without draining. Sampled for 2,4-DCP after 1 hr.

77alhr	36.0	72,	78	2.9	6,			

Table 42e. Transferred adaptation to 2,4-D, followed by 2,4-D / 2,4-DCP mixture.

Key to columns in table: as in Table 42, above.

Perfusion started on 11/8/52 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried February 1952,) and 250 ml. of 100 ppm. 2,4-D solution prepared from the "active" perfusate drained from a 2,4-D enriched perfuser (Table 42c. on 9/8/52,). First sample taken after 1 hr. perfusion.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
0	67.5	56, 61, 73, 83,	19, 19, 22, 30,	4.5,4.5,	100
3	57.2	68, 70, 73, 82,	19, 19, 21, 23,	5, 5,	100
6	58.6	82, 84, 92, 96,	65, 72, 84,104,	31, 46,	10
8b	65.8	91, 99,103,116,	85, 91, 96,102,	81, 91,	1

Day 8. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

8a	67.3	70, 76, 82,101,	18, 19, 21, 21,	3,4.5,	110
10	53.0	104,108,109,117,	98,100,100,109,	79, 90,	<1
12b	57.1	105,107,109,116,	116,116,119,130,	118,118,	<1

Day 12. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

Table 42e. continued.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
12a	57.1	89, 93, 98,104,	23, 25, 25, 26,	5, 7,	85
14	66.9	93, 93, 99, 99,	97,103,103,109,	82, 85,	1
16b	59.3	84, 84,113,117,	78, 79, 88, 88,	69, 78,	1.5

Day 16. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

16a	43.4	58, 69, 74, 78,	25, 30, 30, 30,	7, 7,	70
18	86.2	94, 97, 97,103,	86, 91,100,112,	90,104,	<1
20b	68.8	65, 77, 83, 93,	112,119,119,127,	78, 78,	1

Day 20. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-DCP and 100 ppm. 2,4-DCP. Sampled for 2,4-DCP before adding to the perfuser and for 2,4-D and 2,4-DCP after 1 hr. perfusion.

20a	76.6	46, 57, 68, 72,	14, 15, 17, 21,	2.5, 4,	140
23	72.0	85, 86, 88,118,	28, 28, 28, 33,	5.5, 7,	70
26	63.0	92, 95, 97, 99,	49, 51, 59, 64,	11, 13,	25
29	71.8	78, 78, 84, 86,	47, 49, 50, 60,	11, 11,	30
32	26.4	106,117,121,125,	72, 76, 76, 79,	23, 34,	10
35	49.2	75, 83, 92,114,	73, 83, 88, 96,	31, 33,	7
38b	56.9	100,106,111,123,	83, 84, 86, 92,	48, 53,	3

Day 38. Perfuser drained and refilled as on Day 20.

38a	56.9	53, 55, 60, 70,	19, 20, 25, 30,	3.5,3.5,	120
41	53.3	90, 90, 92,105,	24, 26, 26, 30,	4,5.5,	80
47	49.0	80, 92, 94,108,	102,104,121,123,	90, 96,	<1
50	51.2	111,111,121,123,	125,133,141,145,	88,104,	<1

Day 52. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

52a	55.1	65, 73, 80, 82,	22, 24, 25, 29,	5.5,5.5,	90
54	63.5	77, 85, 88, 96,	77, 82, 87,112,	77, 80,	1
56	55.1	60, 67, 80, 94,	83, 83, 87, 93,	78, 82,	1

Day 58. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

58a	77.5	53, 57, 61, 77,	18, 19, 20, 21,	1.5, 4,	120
60	77.6	85, 85, 88, 89,	85, 90, 93, 99,	32, 37,	6
62	61.6	99,104,123,125,	114,120,123,125,	99,109,	<1
64b	69.6	89,91, 92, 95,	91, 92, 96, 98,	105,105,	<1

Day 64. Perfuser drained and refilled as on Day 20.

64a	75.3	44, 48, 53, 57,	21, 21, 21, 24,	2.5, 4,	105
66	75.3	67, 72, 77,108,	28, 31, 35, 43,	4,6.5,	60
68b	76.5	78, 94, 97,103,	86, 92,106,115,	61, 63,	2

Day 68. 12.5 ml. of 2,000 ppm. 2,4-DCP solution added without draining. Sampled for 2,4-D and 2,4-DCP after 1 hr. perfusion.

Table 42e. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
68a	76.5	93,	94,	98,	98,	89,	93,	96,	107,	71,	82,	1
70b	81.3	87,	91,	94,	100,	87,	94,	94,	113,	68,	105,	<1

Day 70. 12.5 ml. of 2,000 ppm. 2,4-DCP solution added without draining. Sampled for 2,4-D and 2,4-DCP after 1 hr. perfusion.

70	Sample rejected.											
72	80.3	91,	92,	97,	99,	100,	100,	107,	112,	92,	92,	<1

2,4-DCP concentrations during the periods of perfusion with 2,4-D / 2,4-DCP mixture.

Day 20. Perfuser drained and refilled with 2,4-D/2,4-DCP mixture.

A.	G.	H.	A.	G.	H.	A.	G.	H.
20ba	51.4	103,	21	21.6	43,	22b	10.4	21,
20alhr	29.0	58,						

Day 22. 13 ml. of 2,000 ppm. 2,4-DCP solution added without draining. Sampled for 2,4-DCP after 1 hr. perfusion.

22alhr	41.4	83,	24	22	44,	26b	12	24,
23	32	64,	25	15	30,			

Day 26. 13 ml. of 2,000 ppm. 2,4-DCP solution added without draining. Sampled for 2,4-DCP after 1 hr. perfusion.

26alhr	46.5	93,	31	19.4	39,	36	10.8	22,
27	36.4	73,	32	15.5	31,	37	9	18,
28	29.3	59,	33	12.6	25,	38b	10	20,
29	25	50,	34	11	22,			
30	19.9	40,	35	9.5	19,			

Day 38. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 100 ppm. 2,4-DCP.

38ba	63	126,	43	16.8	34,	49	4.6	9,
38alhr	45	90,	44	8.2	16,	50	5	10,
39	29.1	58,	45	4.2	8,	51	6.2	12,
40	33	66,	46	3.1	6,	52	7.5	15,
41	30.6	61,	47	4.5	9,			
42	23	46,	48	3.7	7,			

Day 52. Perfuser drained and refilled with 2,4-D only.

Day 64. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 100 ppm. 2,4-DCP.

64ba	53.4	107,	65	32.5	63,	67	6.9	14,
64alhr	36	72	66	19	38,	68	1.7	3,

Table 42e. continued.

Day 68. 12.5 ml. of 2,000 ppm. 2,4-DCP solution added without draining. Sampled for 2,4-DCP after 1 hr. perfusion.

A.	G.	H.	A.	G.	H.	A.	G.	H.
68alhr	35.0	70,	69	3.6	7,	70b	2.1	4,

Day 70. 12.5 ml. of 2,000 ppm. 2,4-DCP solution added without draining. Sampled for 2,4-DCP after 1 hr. perfusion.

70alhr	36.0	72,	71	2.9	6,	72b	2.8	6,

Day 72. 12.5 ml. of 2,000 ppm. 2,4-DCP solution added without draining. Sampled for 2,4-DCP after 1 hr. perfusion.

72alhr	34.3	69,	73	2.5	5,

Table 42f. Direct perfusion of 2,4-D, followed by a mixture containing 2,4-D and 2,4-DCP.

Key to columns in table: as in Table 42, above.

Perfusion started on 17/6/52 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried February 1952,) and 250 ml. of 100 ppm. 2,4-D solution. First sample taken after 1 hr. perfusion.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
0	44.1	75, 75, 79, 82,	25, 25, 36, 41,	4.5,4.5,	90
3	45.2	66, 71, 93,109,	27, 33, 35, 42,	4.5,6.5,	70
6	47.0	85, 94,100,111,	19, 21, 23, 34,	4, 4,	90
9	59.4	51, 64, 67, 71,	18, 22, 24, 25,	3.5, 5,	105
12	48.3	110,112,131,131,	62, 62, 85, 89,	12, 15,	20
15	41.9	100,117,119,127,	105,136,143,160,	79, 93,	<1
18	47.0	79, 83, 96,151,	100,104,138,168,	55, 79,	2
21b	43.4	129,131,140,159,	101,131,140,178,	95, 99,	1

Day 21. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

21a	43.4	71, 74, 81,118,	28, 28, 37, 39,	4.5, 7,	80
23	54.0	59, 67, 81, 83,	26, 28, 32, 33,	3.5,5.5,	80
25	44.8	100,118,136,154,	80, 87,112,134,	78, 92,	<1
27	48.7	103,103,132,163,	103,113,128,150,	99,128,	<1

Day 29. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

Table 42f. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
29a	52.1	65,	86,	106,	113,	29,	29,	29,	29,	4,	4,	75
31	35.5	70,	86,	102,	127,	51,	51,	59,	76,	8.5,	11,	30
33	44.3	86,	90,	92,	115,	84,	84,	90,	131,	84,	104,	<1

Day 35. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

35a	46.8	81,	88,	92,	115,	24,	30,	30,	30,	4.5,	6.5,	65
37	47.3	53,	53,	68,	76,	85,	87,	114,	116,	17,	34,	10
39	61.9	99,	100,	104,	113,	91,	92,	92,	105,	87,	109,	1

Day 41. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

41a	61.9	60,	66,	71,	75,	21,	23,	23,	28,	5,	8,	95
51b	59.4	106,	110,	116,	120,	103,	105,	113,	128,	111,	113,	<1

Day 51. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

51a	59.4	67,	71,	74,	91,	20,	22,	22,	27,	5,	5,	100
53	51.0	96,	102,	123,	131,	47,	49,	51,	61,	10,	12,	30
55	67.5	73,	77,	83,	92,	90,	92,	92,	93,	77,	113,	<1

Day 57. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 100 ppm. 2,4-DCP. Sampled for 2,4-DCP before adding and for 2,4-D and 2,4-DCP after 1 hr.

57a	55.4	61,	63,	72,	85,	18,	18,	20,	20,	7,	7,	115
59	55.7	68,	75,	83,	97,	25,	25,	27,	31,	5.5,	7,	90

2,4-DCP concentrations in the perfusate during the period of perfusion with 2,4-D / 2,4-DCP mixture.

A.	G.	H.	A.	G.	H.	A.	G.	H.
57ba	51.0	102,	58	26.1	52,	59	20.2	40.
57alhr	34.0	68,						

Table 43. Direct perfusion of 2,5-dichlorophenoxyacetic acid.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Longest roots at nominal concentration of 10 ppm. as % m.c.
G. Indicated 2,5-D concentration (ppm.).

Perfusion started on 28/5/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. 100 ppm. 2,5-D solution.Solution sampled before adding to perfuser.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	G.
0	95.0	77,	80,	82,	86,	25,	25,	27,	28,	12,	18,	100
20	93.7	53,	54,	58,	60,	27,	27,	28,	29,	10,	12,	100
40	105.4	77,	78,	79,	86,	33,	34,	35,	36,	10,	13,	75
60	103.4	77,	79,	82,	85,	33,	35,	38,	48,	7,	7.5,	75
80	100.0	75,	78,	83,	93,	30,	31,	31,	32,	5,	6,	85
90	100.2	69,	70,	73,	78,	28,	29,	31,	33,	6,	7,	90

Table 43a. Direct perfusion of 2,5-dichlorophenoxyacetic acid.

Key to columns in table: as in Table 43, above.

Perfusion started on 16/2/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 100 ppm. 2,5-D solution. First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	86.9	84,	87,	90,	107,	58,	61,	67,	69,	28,	31,	100
5	83.3	62,	72,	77,	90,	63,	67,	71,	80,	29,	34,	90
10	88.2	77,	80,	80,	86,	40,	50,	71,	72,	28,	31,	100
13	82.0	84,	84,	89,	95,	44,	50,	51,	57,	31,	35,	85
16	86.1	89,	90,	92,	100,	53,	55,	60,	66,	26,	27,	115
19	67.8	83,	90,	90,	94,	50,	59,	62,	65,	28,	32,	100
22	91.3	66,	72,	78,	80,	49,	51,	57,	62,	23,	23,	150
25	81.9	74,	77,	79,	82,	38,	39,	45,	52,	22,	23,	160
28	71.0	87,	87,	91,	100,	53,	53,	53,	55,	25,	25,	135
31	77.0	71,	79,	87,	89,	57,	57,	73,	76,	27,	32,	100
34	73.3	57,	58,	83,	92,	54,	57,	61,	62,	27,	30,	105
37	89.4	68,	78,	87,	97,	72,	76,	82,	83,	23,	27,	115
40	84.6	82,	92,	98,	99,	38,	38,	39,	40,	17,	19,	125
43	83.1	65,	70,	71,	106,	61,	65,	67,	82,	22,	22,	100

Table 43a. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	E.
46	72.1	83,	83,	87,	90,	61,	61,	62,	86,	25,	31,	110
51	81.9	89,	93,	94,	100,	71,	74,	77,	78,	27,	27,	110
56	77.9	69,	72,	94,	108,	50,	51,	53,	55,	27,	30,	105
66	76.7	83,	90,	103,	116,	51,	57,	59,	69,	22,	23,	110
76	81.4	95,	95,	99,	101,	58,	60,	64,	75,	25,	27,	125
86	80.7	101,	105,	106,	144,	87,	87,	88,	94,	29,	31,	90
96	88.3	95,	96,	98,	102,	70,	77,	83,	83,	34,	41,	65
106	93.9	94,	103,	103,	104,	80,	83,	85,	88,	32,	42,	65
116	91.9	91,	92,	92,	97,	68,	72,	72,	82,	36,	41,	60

Table 44. Direct perfusion of 2,5-dichlorophenoxyacetic acid.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Longest roots at nominal concentration of 10 ppm. as % m.c.
G. Indicated 2,5-D concentration (ppm.).

Perfusion started on 16/2/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 10 ppm. 2,5-D solution. First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
0	86.9	95,	97,	99,	101,	41,	49,	52,	69,	16,	20,	10.0
5	83.3	80,	86,	93,	95,	68,	69,	69,	72,	20,	31,	13.0
10	88.2	93,	97,	99,	105,	76,	77,	79,	88,	24,	24,	10.0
13	82.0	83,	83,	87,	90,	56,	61,	71,	71,	35,	38,	12.5
16	86.1	80,	84,	102,	109,	72,	73,	81,	82,	27,	31,	9.0
19	67.8	85,	88,	91,	106,	60,	66,	72,	78,	22,	31,	12.5
22	91.3	85,	90,	95,	103,	61,	62,	72,	98,	24,	25,	12.5
25	81.9	67,	70,	70,	78,	52,	54,	55,	63,	27,	31,	10.0
28	71.0	100,	106,	108,	110,	67,	69,	73,	93,	34,	37,	12.5
31	77.0	70,	80,	87,	93,	65,	69,	74,	83,	22,	23,	11.5
34	73.3	60,	61,	62,	67,	57,	61,	68,	71,	24,	27,	12.5
37	89.4	87,	89,	90,	90,	66,	67,	68,	77,	26,	29,	11.0
40	84.6	86,	91,	91,	101,	59,	59,	64,	72,	34,	37,	12.5
43	83.1	66,	67,	74,	82,	67,	70,	71,	79,	28,	29,	11.0
46	72.1	54,	54,	57,	69,	36,	39,	46,	46,	31,	37,	7.5
51	81.9	66,	67,	82,	94,	62,	70,	82,	95,	24,	26,	11.0
56	77.9	79,	81,	89,	94,	59,	68,	72,	90,	32,	33,	11.0
66	76.7	89,	95,	96,	100,	65,	70,	77,	86,	34,	35,	10.0
76	81.4	67,	80,	87,	87,	70,	70,	82,	96,	23,	28,	12.0
86	80.7	108,	110,	110,	113,	93,	95,	100,	110,	36,	38,	5.5
96	88.3	90,	94,	96,	102,	88,	90,	96,	97,	31,	34,	9.0
106	93.9	88,	92,	92,	94,	80,	81,	84,	86,	36,	37,	6.0
116	91.9	103,	103,	111,	115,	83,	84,	87,	88,	45,	46,	4.0
126	101.7	82,	86,	89,	90,	88,	89,	98,	102,	60,	66,	1.5
136b	97.5	103,	106,	106,	117,	88,	91,	92,	99,	84,	85,	0.5

Perfuser drained and refilled with 250 ml. of 10 ppm. 2,5-D solution. Perfuser re-started, sampled after 1 hr. Day 136.

136a	97.5	85,	90,	92,	100,	63,	69,	77,	78,	32,	36,	11.0
146,	95.3	95,	98,	101,	103,	89,	90,	91,	103,	40,	40,	5.0
156	102.6	95,	97,	100,	105,	93,	95,	95,	100,	54,	56,	2.5
166	98.4	91,	93,	100,	101,	90,	90,	90,	90,	43,	44,	4.0
171b	93.9	99,	101,	101,	102,	94,	95,	98,	103,	41,	42,	4.5

Table 44. continued.

Day 171. Perfuser drained and refilled with 250 ml. of 10 ppm. 2,5-D solution. Perfuser re-started, sampled after 1 hr.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	G.
171a	93.9	90,	90,	92,	103,	18,	20,	26,	31,	3,	4.5,	9.0
173	93.9	91,	95,	100,	112,	21,	22,	23,	27,	4.5,	4.5,	7.5
175	93.9	78,	78,	78,	89,	28,	30,	31,	34,	5.5,	6.5,	7.0
179	99.8	87,	87,	88,	99,	24,	25,	26,	27,	4,	5,	8.5
185	96.3	79,	82,	82,	93,	35,	37,	39,	40,	6,	6,	5.0
191	100.2	92,	92,	95,	97,	36,	38,	38,	38,	6,	7,	5.0
197	100.8	88,	89,	93,	97,	87,	88,	90,	94,	45,	45,	0.5

Table 44a. Direct perfusion of 2,5-dichlorophenoxyacetic acid.

Key to columns in table: as in Table 44, above.

Perfusion started on 28/5/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 10 ppm. 2,5-D solution. Solution sampled before adding to perfuser.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	G.
0	95.0	80,	82,	94,	99,	29,	33,	35,	37,	12,	15,	8.0
20	93.7	58,	62,	63,	67,	21,	22,	25,	31,	13,	15,	12.0
40	105.4	71,	74,	74,	77,	29,	32,	34,	34,	7.5,	8.5,	10.0
60	103.4	82,	82,	83,	90,	29,	29,	37,	38,	8.5,	9.5,	10.0
80	100.0	86,	89,	93,	95,	36,	37,	41,	42,	8,	13,	10.0
90	100.2	94,	97,	100,	101,	47,	49,	50,	50,	15,	18,	5.0
96	100.8	92,	92,	94,	102,	42,	45,	48,	51,	13,	15,	3.5

Table 45. Direct perfusion of 3,4-dichlorophenoxyacetic acid.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.1 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
E. Longest roots at nominal concentration of 10 ppm. as % m.c.
F. Indicated 3,4-D concentration in the perfusate (ppm.).

Perfusion started on 6/7/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 100 ppm. 3,4-D solution. First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	95.3	95,	96,	101,	102,	38,	41,	48,	49,	5,	9,	65
6	95.3	91,	91,	91,	103,	39,	41,	42,	46,	3,	5,	80
11	95.9	93,	95,	96,	104,	38,	38,	39,	43,	8,	9,	85
16	102.9	86,	87,	88,	94,	37,	38,	42,	42,	6,	12,	105
21	103.4	89,	90,	90,	92,	39,	39,	40,	45,	6,	8,	115
31	100.0	86,	88,	89,	90,	38,	38,	43,	44,	5,	8,	115
41	100.0	86,	87,	88,	98,	34,	37,	38,	39,	6,	10,	95
51	100.2	85,	86,	88,	96,	40,	44,	44,	50,	5,	6,	95
56	99.1	86,	86,	88,	95,	37,	38,	38,	53,	8,	10,	80

Table 46. Direct perfusion of 3,4-dichlorophenoxyacetic acid.

Key to columns, and details of perfuser set up, as in Table 45, above, except that the initial 3,4-D concentration was 10 ppm.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	95.3	91,	94,	96,	97,	42,	43,	45,	52,	5,	7,	9.0
6	95.3	85,	87,	97,	107,	39,	39,	40,	42,	3,	5,	9.0
11	95.9	91,	91,	92,	102,	35,	38,	41,	42,	8,	8,	10.5
16	102.9	86,	86,	90,	91,	29,	33,	33,	33,	6,	7,	11.0
21	103.4	82,	83,	88,	90,	31,	33,	41,	45,	4,	5,	11.0
31	100.0	88,	89,	92,	97,	32,	38,	44,	46,	4,	5,	9.0
41	100.0	93,	99,	101,	111,	41,	43,	49,	50,	7,	8,	6.0
51	100.2	93,	94,	105,	107,	54,	58,	62,	75,	9,	11,	4.0
56	99.1	97,	97,	98,	98,	55,	56,	56,	64,	10,	11,	5.0

Table 47. Direct perfusion of 2,4,5-trichlorophenoxyacetic acid.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.1 ppm. as percentage of mean control.
D. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
E. Longest roots at nominal concentration of 10 ppm. as % m.c.
F. Indicated 2,4,5-T concentration in the perfusate (ppm.).

Perfusion started on 20/12/51 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried June 1951,) and 250 ml. of 100 ppm. 2,4,5-T solution. Solution (common with that in Table 47a,) sampled before adding to the perfuser.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	52.3	67,	72,	80,	90,	18,	23,	25,	36,	4,	5.5,	60
10	52.3	63,	69,	88,	93,	15,	25,	29,	31,	4,	5.5,	65
20	71.6	63,	64,	67,	70,	31,	34,	39,	50,	4,	5.5,	55
30	59.0	76,	78,	78,	94,	19,	19,	20,	22,	10,	10,	60
40	86.6	47,	49,	72,	79,	35,	44,	47,	59,	6,	8,	40
50	63.8	61,	69,	92,	107,	25,	38,	38,	41,	20,	20,	30
60	83.8	66,	72,	73,	86,	55,	62,	73,	88,	19,	21,	8
70	67.4	77,	95,	108,	123,	50,	56,	71,	97,	16,	24,	7
80	67.5	85,	90,	101,	107,	53,	57,	59,	70,	22,	22,	11
90	72.5	105,	109,	123,	123,	80,	87,	97,	102,	39,	46,	2.5

Day 100. Perfusate volume made up to approximately 250 ml. of 100 ppm. 2,4,5-T without draining. Perfuser sampled after 1 hr.

100a	69.6	53,	84,	85,	92,	24,	27,	35,	40,	7,	8.5,	40
110	77.6	81,	88,	96,	108,	18,	19,	28,	30,	4,	5,	50
130	69.3	57,	62,	67,	83,	24,	24,	24,	37,	6,	6,	55
140	62.5	90,	95,	109,	109,	19,	22,	30,	32,	3,	5,	55
150	54.4	81,	90,	90,	97,	16,	18,	22,	31,	3.5,	3.5,	65
160	52.3	92,	96,	98,	103,	33,	34,	36,	38,	4,	5.5,	35
170	46.3	87,	87,	110,	132,	32,	32,	39,	43,	4.5,	6.5,	40
180	44.1	70,	77,	82,	86,	48,	66,	68,	73,	4.5,	7,	40

Table 47a. Direct perfusion of 2,4,5-trichlorophenoxyacetic acid.

Key to columns in table: as in Table 47, above.
Details of perfusion set-up: as in Table 47, above.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	52.3	67,	72,	80,	90,	18,	23,	25,	36,	4,	5.5,	60

Table 47a. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
10	52.3	57,	69,	74,	82,	19,	21,	25,	29,	4,	4,	65
20	71.6	56,	60,	67,	74,	15,	22,	24,	32,	7.5,	9,	50
30	52.0	114,	116,	133,	144,	37,	38,	41,	71,	7,	8.5,	30
40	86.6	50,	58,	66,	72,	31,	36,	44,	64,	11,	14,	20
50	63.8	102,	103,	108,	114,	42,	49,	69,	102,	22,	42,	4
60	83.8	84,	90,	106,	116,	56,	75,	81,	86,	15,	33,	7
70	67.4	74,	79,	112,	123,	55,	71,	74,	82,	13,	25,	9
80	67.5	83,	86,	95,	110,	52,	55,	56,	61,	21,	22,	13
90	72.5	90,	98,	100,	109,	65,	69,	76,	80,	23,	26,	6
Day 100. Perfusate volume made up to approximately 250 ml. of 100 ppm. 2,4,5-T without draining. Perfuser sampled after 1 hr.												
100a	69.6	49,	58,	71,	71,	20,	23,	24,	26,	3,	4.5,	80
110	77.6	65,	81,	94,	103,	13,	14,	17,	18,	2.5,	2.5,	110
130	69.3	87,	97,	97,	103,	13,	17,	20,	20,	3,	4.5,	80
140	62.5	38,	38,	61,	120,	24,	30,	32,	37,	3,	5,	80
150	54.4	85,	92,	94,	114,	14,	15,	26,	33,	3.5,	5.5,	70
160	52.3	42,	44,	46,	48,	29,	31,	33,	42,	4,	4,	80
170	46.3	65,	76,	80,	82,	15,	17,	20,	39,	2,	6.5,	75
180	44.1	55,	61,	73,	84,	39,	43,	45,	52,	7,	9,	30

Table 48. Direct perfusion of MCPA, followed by 2,4-D.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.001 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.01 ppm. as % m.c.
E. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
F. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
G. Indicated MCPA concentration in the perfusate (ppm.).
H. Indicated 2,4-D concentration in the perfusate (ppm.).

Perfusion started on 22/12/50 with 50 gm. of soil (1 to 4 mm., Sussex Lodge soil, dried September 1950,) and 200 ml. of old stock MCPA solution (apparently well below nominal strength,). Solution sampled before adding to the perfuser and after 2 hr. perfusion.

A.	B.	E.	E.	E.	E.	D.	D.	D.	D.	C.	C.	G.
0ba	33.8	36,	41,	44,	62,	89,	98,	98,	101,	113,	124,	25
0a2hr	33.8	33,	44,	59,	62,	83,	107,	107,	131,	110,	118,	20
11	33.8	39,	44,	65,	74,	74,	95,	98,	104,	113,	118,	15
18	31.9	44,	44,	53,	56,	81,	106,	119,	122,	119,	160,	25
32	32.8	34,	49,	52,	52,	76,	79,	85,	94,	91,	113,	20

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	G.
39	39.4	61,	69,	71,	84,	23,	30,	41,	48,	5,	5,	30
46	38.1	34,	42,	63,	97,	24,	29,	31,	34,	2.5,	5,	35
53	39.4	64,	66,	74,	84,	36,	38,	38,	46,	7.5,	7.5,	25
60	38.0	58,	61,	61,	61,	26,	29,	32,	37,	2.5,	5.5,	40

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
64	39.8	48,	48,	78,	93,	45,	45,	45,	60,	33,	35,	50
67	22.1	84,	106,	106,	120,	93,	115,	115,	124,	44,	57,	25
70	31.1	93,	96,	109,	161,	67,	71,	71,	80,	35,	39,	30

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	G.
73	30.0	47,	63,	67,	80,	40,	43,	43,	43,	3,	7,	25

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
75	30.1	80,	80,	107,	110,	87,	90,	120,	133,	27,	63,	25

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	G.
77	37.6	56,	72,	77,	82,	29,	29,	32,	35,	3,	5,	40
79	23.5	72,	76,	85,	94,	43,	47,	55,	72,	4,	8.5,	30
81	35.2	58,	67,	72,	78,	33,	36,	44,	64,	5.5,	5.5,	25
83	30.0	67,	70,	80,	87,	33,	40,	43,	60,	7,	7,	25
85	29.3	58,	72,	75,	106,	41,	48,	58,	61,	7,	7,	25
87	35.4	107,	111,	116,	122,	65,	68,	68,	74,	8.5,	8.5,	10

Table 48. continued.

Day 103. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	G.
103a	35.4	82,	97,	99,	113,	25,	25,	26,	31,	3,	3,	50
105	35.4	85,	88,	99,	119,	25,	28,	31,	42,	5.5,	5.5,	45
107	39.5	58,	78,	90,	96,	25,	25,	28,	46,	5,	5,	50
109	29.1	41,	52,	58,	79,	34,	34,	38,	65,	3.5,	7,	30
111	29.1	65,	72,	76,	110,	34,	41,	45,	48,	7,	7,	25
113	36.8	65,	68,	90,	101,	44,	46,	52,	55,	7,	9,	20

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
115	36.8	82,	95,	109,	112,	55,	60,	79,	98,	30,	35,	35
117	36.8	106,	112,	114,	117,	76,	79,	82,	82,	27,	52,	30
119	31.3	77,	80,	83,	87,	61,	64,	64,	71,	51,	61,	15

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	G.
123	35.8	59,	75,	78,	78,	75,	75,	78,	78,	8.5,	11,	10
125	17.6	120,	125,	130,	131,	68,	74,	85,	108,	17,	23,	7
126	36.7	69,	69,	74,	91,	53,	61,	61,	64,	8,	13,	12
129	33.1	94,	100,	100,	151,	60,	69,	73,	82,	3,	12,	10
131	29.3	133,	143,	147,	167,	69,	78,	99,	102,	24,	34,	5
133	43.0	93,	102,	104,	121,	65,	79,	84,	86,	30,	35,	3.5
135	46.2	95,	100,	106,	115,	63,	76,	87,	100,	48,	50,	2
136	35.2	68,	94,	97,	111,	85,	110,	120,	182,	88,	91,	<1
138	35.5	110,	110,	133,	138,	96,	104,	107,	130,	79,	110,	<1
139	35.7	92,	95,	95,	98,	104,	115,	115,	118,	101,	115,	<1

Day 141. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 3 hrs.

141a	34.8	69,	83,	86,	101,	26,	29,	32,	35,	6,	8.5,	40
143	43.0	60,	70,	70,	93,	28,	37,	49,	56,	7,	7,	25
145	34.5	87,	96,	99,	104,	49,	52,	55,	58,	6,	6,	20
147	45.3	55,	66,	82,	95,	38,	38,	44,	49,	4.5,	4.5,	25
149	45.3	71,	75,	80,	80,	35,	42,	53,	55,	4.5,	6.5,	30
151	41.8	120,	124,	129,	136,	36,	41,	41,	53,	12,	17,	12
153	34.1	97,	102,	102,	111,	62,	68,	70,	79,	20,	50,	10
155	42.4	97,	104,	118,	118,	71,	71,	73,	90,	28,	35,	4
157	40.2	90,	95,	104,	130,	90,	92,	97,	107,	60,	70,	1
159	42.8	68,	94,	96,	117,	82,	82,	89,	94,	100,	117,	<1

Day 161. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

161a	45.2	69,	69,	71,	77,	27,	27,	33,	33,	2,	4.5,	50
163	45.2	66,	68,	86,	88,	29,	29,	40,	42,	4.5,	4.5,	45
165	38.8	82,	85,	77,	98,	39,	41,	46,	46,	5,	5,	25
167	39.8	96,	108,	111,	130,	58,	58,	63,	83,	15,	25,	15
169	40.7	101,	101,	108,	135,	76,	79,	81,	81,	34,	44,	5
171	40.7	81,	91,	93,	116,	74,	86,	86,	96,	91,	116,	<1

Table 48. continued.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	G.
173	36.6	96,	107,	107,	110,	90,	90,	90,	96,	96,	104,	<1

Day 175. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

175a	36.6	57,	74,	76,	87,	23,	25,	26,	27,	4,	5.5,	50
179	36.6	90,	90,	93,	96,	49,	52,	52,	60,	8,	11,	20
183	36.6	96,	96,	98,	108,	96,	98,	108,	144,	66,	74,	1
192	43.7	92,	96,	98,	132,	92,	94,	101,	106,	94,	124,	<1

Day 197. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

197a	40.7	84,	91,	103,	103,	39,	44,	44,	57,	5,	5,	40
199	37.7	61,	67,	67,	72,	45,	45,	51,	51,	2.5,	5.5,	20
201	36.7	74,	77,	85,	85,	49,	49,	55,	55,	5.5,	5.5,	15
203	35.2	80,	80,	85,	105,	51,	57,	57,	65,	5.5,	5.5,	15
205	42.8	70,	77,	80,	84,	44,	47,	54,	59,	9.5,	12,	15
207	34.8	66,	69,	69,	72,	52,	52,	60,	72,	8.5,	8.5,	20
209	37.6	72,	72,	75,	75,	59,	59,	61,	67,	13,	16,	10
212	30.9	62,	65,	68,	71,	100,	104,	104,	107,	74,	84,	<1
214	33.5	120,	123,	128,	144,	99,	99,	99,	114,	87,	128,	<1

Day 217. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

217a	38,37	66,	71,	81,	94,	32,	36,	47,	50,	3,	5,	50
220	41.3	68,	73,	73,	80,	41,	46,	49,	49,	5,	5,	40
222	42.8	66,	66,	68,	87,	42,	47,	49,	58,	4.5,	7,	30
224	43.6	55,	71,	71,	73,	46,	46,	48,	53,	9,	9,	20
226	40.1	82,	95,	102,	120,	72,	77,	80,	85,	37,	45,	5
228	43.5	94,	97,	106,	110,	92,	94,	99,	108,	91,	85,	<1
230	64.2	96,	101,	106,	129,	106,	112,	112,	131,	97,	114,	<1

Day 232. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

232a	62.2	97,	98,	104,	104,	24,	29,	32,	37,	3,	5,	40
235	49.7	135,	141,	149,	172,	34,	38,	40,	40,	4,	4,	25
238	67.4	98,	99,	101,	110,	47,	56,	58,	58,	6,	7.5,	15
239	67.3	89,	94,	98,	104,	79,	89,	95,	110,	88,	93,	<1

Day 242. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

242a	58.8	56,	87,	98,	98,	22,	24,	26,	29,	3.5,	3.5,	55
247	67.2	97,	97,	98,	101,	82,	82,	97,	103,	52,	52,	2
249	72.8	100,	103,	104,	118,	85,	87,	92,	103,	81,	83,	<1

Day 250. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

Table 48. continued.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	G.
250a	74.8	67,	68,	84,	90,	21,	25,	27,	39,	2.5,	4,	50
252	70.1	86,	89,	94,	107,	27,	29,	30,	33,	4,	4,	40
254	56.4	92,	96,	98,	114,	89,	106,	110,	119,	78,	83,	1
256	60.1	108,	113,	113,	125,	105,	110,	110,	130,	110,	138,	<1

Day 258. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

258	51.6	60,	62,	74,	84,	27,	29,	29,	31,	6,	6,	50
264	55.0	118,	124,	124,	140,	82,	82,	98,	146,	66,	77,	1
266b	56.0	102,	107,	118,	125,	116,	125,	125,	136,	79,	106,	<1

Day 266. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

266a	56.0	61,	68,	75,	95,	21,	27,	29,	32,	3.5,	6,	50
272	76.3	87,	89,	89,	100,	85,	85,	89,	119,	51,	75,	1
274b	68.8	54,	55,	68,	93;	80,	86,	87,	95,	79,	80,	<1

Day 274. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

274a	57.5	75,	78,	82,	96,	21,	23,	30,	33,	3.5,	5,	50
279	73.0	88,	93,	110,	116,	55,	58,	58,	64,	9.5,	14,	15
281b	46.0	94,	102,	110,	120,	65,	70,	89,	104,	63,	72,	1

Day 281. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

281a	46.0	94,	108,	118,	128,	37,	39,	39,	41,	4.5,	4.5,	40
285	67.6	93,	104,	105,	110,	82,	86,	89,	98,	62,	74,	1
287	69.1	94,	96,	107,	107,	90,	90,	98,	98,	97,	113,	<1

Day 289. Perfuser drained and refilled with 250 ml. of 10 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

289a	51.6	85,	91,	93,	95,	66,	68,	74,	84,	23,	27,	8.0
295	59.3	100,	104,	104,	120,	67,	71,	81,	106,	32,	40,	0.3
297	67.7	77,	92,	99,	108,	83,	96,	104,	106,	68,	82,	<0.1
299	69.9	103,	106,	114,	133,	113,	116,	133,	134,	129,	150,	<0.1

Day 301. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

301a	36.5	90,	101,	118,	129,	36,	44,	47,	47,	5.5,	5.5,	20
305	51.7	99,	126,	128,	134,	45,	46,	52,	54,	6,	9.5,	20
307	53.3	79,	92,	100,	139,	81,	83,	87,	88,	70,	102,	<1
309	52.3	84,	92,	96,	103,	67,	92,	107,	128,	81,	88,	<1

Day 310. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

Table 48. continued.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	G.
310a	49.8	60,	70,	76,	82,	24,	26,	28,	28,	4,	6,	45
314	60.7	76,	79,	83,	89,	63,	66,	66,	76,	76,	79,	1
316	66.8	102,	114,	117,	132,	79,	87,	90,	93,	90,	94,	<1

Day 317. Perfuser drained and refilled with 250ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

317a	55.8	74,	75,	83,	91,	16,	16,	17,	27,	3.5,	3.5,	80
320	71.5	73,	74,	84,	85,	60,	60,	67,	72,	10,	17,	15
322	70.7	68,	92,	93,	98,	68,	69,	72,	74,	79,	88,	<1
324	62.8	107,	107,	111,	120,	96,	102,	108,	108,	94,	107,	<1

Day 325. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

325a	67.0	69,	73,	76,	78,	16,	19,	19,	19,	4.5,	4.5,	70
328	79.1	79,	81,	91,	91,	58,	61,	74,	88,	14,	18,	10
330	75.2	97,	100,	103,	104,	83,	87,	98,	101,	72,	76,	1
332												

Day 335. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

335a	62.2	82,	82,	90,	97,	23,	26,	27,	29,	3,	5,	55
338	55.9	116,	122,	142,	145,	84,	86,	93,	100,	52,	57,	2
340	68.3	87,	88,	97,	108,	59,	62,	73,	81,	84,	98,	<1

Day 342. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

Key to columns in remainder of table: as above, with the addition of I. Longest roots at nominal concentration of 10 ppm. as percentage mean control.

A.	B.	E.	E.	E.	E.	F.	F.	F.	F.	I.	I.	H.
342a	66.0	19,	20,	27,	33,	3,	3,	3,	3,	0,	1.5,	1,000
345	58.6	21,	22,	29,	31,	5,	7,	7,	8.5,	2,	2,	900
347	67.6	22,	25,	25,	28,	3,	3,	3,	3,	1.5,	1.5,	850
349	70.7	18,	21,	23,	24,	3,	3,	4,	4,	0,	1,	1,000
351	49.8	36,	40,	46,	46,	5,	6,	8,	9,	1,	2,	500
354	75.4	43,	45,	51,	55,	16,	20,	20,	28,	2.5,	4,	120
357	67.8	46,	49,	50,	55,	9,	10,	11,	15,	1.5,	1.5,	300
360	48.8	37,	47,	51,	62,	6,	8,	12,	14,	1,	2,	350
373	73.3	57,	61,	63,	64,	7,	8,	15,	18,	3,	4.5,	200
376	81.3	37,	46,	59,	83,	19,	19,	23,	28,	3.5,	6,	100
379	77.7	83,	90,	93,	95,	58,	63,	69,	76,	7,	8,	20
382b	72.0	74,	77,	90,	92,	82,	85,	89,	97,	81,	95,	<10

Table 48. continued.

Day 382. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	H.
382a	72.0	63,	71,	74,	93,	28,	31,	32,	33,	4,	5.5,	700
387	52.8	78,	78,	84,	114,	28,	30,	30,	32,	9.5,	12,	700
389	66.3	72,	74,	86,	107,	23,	26,	29,	33,	4.5,	7.5,	800
392	66.3	53,	56,	78,	81,	27,	32,	33,	39,	7.5,	9,	500
395	51.2	70,	78,	78,	88,	51,	72,	78,	86,	15,	31,	120
398	49.9	110,	110,	112,	118,	112,	116,	122,	124,	90,	120,	<10

Table 49. Transfer of MCPA adaptation to crushed flower-pot.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Indicated MCPA concentration in the perfusate (ppm.).

Perfusion started on 25/9/52 with 50 gm. of 2 to 4 mm., acid-washed, crushed flower-pot and 250 ml. of 100 ppm. MCPA solution prepared from the perfusate drained from two MCPA enriched perfusers (Tables 51a, 57b,) on 25/9/52. Sampled after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	49.0	51,	51,	59,	76,	18,	24,	24,	27,	2.5,	3.5,	70
6	49.0	53,	55,	55,	57,	18,	24,	27,	27,	2,	2,	75
15	67.4	45,	59,	59,	65,	12,	18,	21,	24,	2,	3,	75
18	64.8	43,	43,	45,	54,	15,	17,	22,	23,	1.5,	3,	80
21	76.5	61,	63,	69,	78,	21,	21,	22,	26,	1.5,	2.5,	80
24	76.3	42,	46,	55,	67,	16,	20,	20,	24,	2.5,	2.5,	75
27	80.3	58,	66,	67,	91,	15,	20,	25,	27,	2.5,	3.5,	70
30	76.6	55,	55,	56,	63,	18,	19,	20,	21,	4,	6.5,	75
33	65.4	46,	50,	60,	72,	14,	18,	21,	26,	1.5,	3,	75
36	68.6	51,	54,	63,	67,	18,	19,	19,	20,	1.5,	3,	80
39	65.2	43,	46,	69,	71,	18,	21,	25,	26,	3,	3,	60
42	50.0	56,	56,	58,	66,	20,	20,	20,	30,	6,	8,	70
45b	65.0	75,	75,	82,	83,	20,	20,	25,	26,	3,	4.5,	75

Day 45. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution prepared from the "active" perfusate drained from a MCPA enriched perfuser (Table 60a,) on 9/11/52. Perfuser re-started, sampled after 1 hr.

45a	60.6	56,	61,	63,	71,	20,	21,	23,	30,	3.5,	5,	70
48	64.7	59,	60,	66,	70,	26,	28,	36,	43,	4.5,	4.5,	50
51	74.4	50,	52,	55,	57,	22,	26,	31,	34,	2.5,	2.5,	50
54	75.1	68,	69,	71,	71,	25,	27,	31,	31,	5.5,	9.5,	40
57	66.5	81,	87,	90,	105,	30,	33,	33,	41,	4.5,	7.5,	40
60	79.2	93,	97,	101,	112,	37,	40,	43,	45,	5,	5,	30
63	77.9	91,	95,	101,	109,	62,	82,	92,	94,	9,	10,	10
66	64.0	100,	102,	102,	105,	91,	92,	100,	110,	58,	81,	1
69	45.3	93,	101,	113,	117,	71,	71,	71,	84,	71,	82,	<1
72b	68.0	96,	102,	107,	109,	78,	78,	87,	97,	75,	87,	<1

Day 72. 1% stock MCPA solution and distilled water added to the perfuser without draining, to make the perfusate 250 ml. of approximately 100 ppm. MCPA. Perfuser re-started, sampled after 1 hr.

72a	62.9	56,	60,	60,	65,	13,	16,	16,	18,	1.5,	3,	95
75	79.7	49,	49,	60,	73,	17,	21,	21,	23,	1.5,	2.5,	70

Table 49. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
78	85.1	60,	68,	73,	78,	14,	14,	14,	16,	1,	2.5,	100
81	65.0	68,	71,	75,	81,	20,	22,	22,	28,	1.5,	3,	70
83	77.1	60,	61,	66,	74,	22,	26,	29,	32,	4,	4,	50
104b	66.6	95,	99,	101,	101,	63,	66,	68,	75,	27,	30,	5

Day 104. 1% stock MCPA solution and distilled water added to the perfuser without draining, to make the perfusate 250 ml. of approximately 100 ppm. MCPA. Perfuser re-started, sampled after three days.

107	65.3	49,	57,	60,	70,	14,	15,	17,	19,	1.5,	4.5,	90
110	68.5	54,	60,	61,	67,	19,	19,	21,	22,	1.5,	3,	70
113	71.0	60,	62,	63,	65,	27,	30,	30,	38,	7,	8.5,	40
116	76.0	59,	59,	74,	83,	68,	71,	101,	101,	70,	78,	1
119	81.3	73,	73,	89,	92,	80,	85,	91,	91,	75,	79,	<1
122	75.3	77,	78,	82,	85,	93,	95,	97,	101,	80,	85,	<1

Day 125, Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

125a	76.6	46,	53,	53,	60,	9,	11,	15,	16,	1.5,	4,	100
130	87.8	56,	59,	65,	77,	10,	11,	13,	15,	1,	2.5,	100
133	87.1	56,	60,	70,	88,	15,	15,	15,	16,	3.5,	3.5,	90
136	91.0	76,	82,	93,	100,	41,	42,	48,	49,	11,	11,	30
139	83.8	68,	68,	70,	105,	74,	80,	83,	98,	43,	76,	1.5
142	76.3	90,	90,	94,	99,	73,	81,	95,	101,	44,	46,	2
145b	81.1	87,	87,	89,	94,	79,	80,	86,	102,	62,	64,	1

Day 145. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

145a	81.1	39,	46,	47,	65,	12,	15,	15,	15,	2.5,	2.5,	100
148	82.4	45,	46,	48,	62,	18,	19,	22,	29,	3.5,	5,	65
151	83.5	57,	57,	63,	75,	19,	22,	28,	29,	3.5,	3.5,	50
154	85.3	76,	76,	79,	88,	61,	65,	65,	79,	17,	21,	10
157	86.1	82,	86,	89,	100,	92,	92,	102,	108,	92,	114,	<1
160b	81.2	90,	93,	102,	108,	80,	82,	105,	111,	69,	114,	<1

Day 160. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. MCPA and 1,000 ppm. sodium azide. Perfuser re-started, sampled after 1 hr.

160a	81.2	48,	52,	52,	53,	12,	13,	14,	15,	1,	2.5,	115
166	81.6	29,	35,	35,	49,	11,	13,	14,	15,	1,	2.5,	110
172	78.7	30,	32,	36,	48,	11,	12,	13,	22,	2.5,	2.5,	120
178	72.5	44,	45,	51,	55,	11,	11,	13,	14,	1.5,	1.5,	125
184	77.8	44,	46,	53,	55,	10,	11,	13,	14,	1.5,	2.5,	125

Table 50. Transferred adaptation to MCPA, followed by 3,4-D.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Longest roots at nominal concentration of 10 ppm. as % m.c.
G. Indicated MCPA concentration in the perfusate (ppm.).
H. Indicated 3,4-D concentration in the perfusate (ppm.).

Perfusion started on 14/6/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 100 ppm. MCPA solution. This solution prepared from the combined "active" perfusates drained from two MCPA enriched perfusers (Tables 52 and 58a,) on 12/6/53. First sample after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
0	91.7	72,	73,	76,	77,	13,	13,	14,	18,	2,	3.5,	115
10	100.1	76,	79,	86,	91,	19,	19,	23,	26,	3,	3,	90
20	99.3	79,	84,	91,	92,	29,	30,	32,	33,	5,	5,	50
30	97.3	101,	102,	105,	110,	98,	104,	105,	110,	94,	111,	<1

Day 35. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

35a	105.1	80,	80,	81,	83,	17,	18,	19,	20,	2,	3,	95
38	102.9	86,	88,	90,	100,	32,	39,	39,	46,	3,	4,	45
41	100.1	100,	100,	107,	110,	101,	103,	105,	110,	89,	93,	<1

Day 44. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

44a	101.2	87,	87,	89,	90,	21,	23,	24,	27,	3,	3,	80
47	96.3	86,	86,	87,	88,	46,	46,	49,	49,	8.5,	9.5,	30
50	99.4	96,	99,	104,	115,	101,	102,	104,	106,	102,	106,	<1

Day 53. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

53a	102.3	80,	85,	86,	89,	15,	16,	16,	17,	2,	3,	110
55	102.3	85,	85,	87,	87,	21,	22,	23,	25,	4,	4,	55
57b	102.3	100,	101,	101,	115,	91,	93,	94,	105,	98,	100,	<1

Day 57. Perfuser drained and refilled with 250 ml. of 10 ppm. 3,4-D solution. Perfuser re-started, sampled after 1 hr.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	H.
57a	100.3	93,	94,	97,	97,	45,	47,	52,	58,	11,	14,	6.0

Table 50. continued.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	H.
60	101.4	97,	98,	99,	102,	99,	100,	102,	107,	78,	82,	2.0
63	100.0	103,	103,	104,	110,	82,	90,	92,	98,	73,	77,	2.0

Day 66. Perfuser drained and refilled with 250 ml. of 10 ppm. 3,4-D solution. Perfuser re-started, sampled after 1 hr.

66a	96.3	88,	89,	92,	98,	38,	40,	45,	46,	7.5,	8.5,	7.5
69	105.1	82,	83,	86,	87,	38,	38,	38,	41,	7.5,	10,	7.5
72	100.6	92,	94,	95,	96,	45,	47,	51,	59,	13,	15,	5.0
75	99.1	95,	96,	97,	97,	56,	57,	60,	62,	21,	27,	4.0
78	100.8	97,	97,	98,	101,	68,	73,	73,	78,	21,	27,	2.5

Table 50a. Attempted transfer of MCPA adaptation.

Key to columns in table: as in Table 50, above.

Perfusion started on 14/6/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 100 ppm. MCPA solution. This solution was prepared from the combined "active" perfusates drained from two MCPA enriched perfusers (Tables 52 and 58a,) on 12/6/53. Solution boiled gently for 30 mins., then cooled before adding to the perfuser. First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
0	91.7	66,	69,	69,	70,	17,	18,	19,	22,	1,	2,	85
10	100.1	77,	78,	81,	82,	20,	24,	25,	28,	2,	3,	75
20	99.3	76,	81,	86,	86,	17,	18,	20,	21,	2,	3,	75
30	97.3	79,	81,	85,	97,	15,	18,	20,	26,	2,	3,	75

Table 51. Transferred adaptation to MCPA, followed by 2-CPA.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean comtrol.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Longest roots at nominal concentration of 10 ppm. as % m.c.
G. Indicated MCPA concentration in the perfusate (ppm.).
H. Indicated 2-CPA concentration in the perfusate (ppm.).

Perfusion started on 23/8/52 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried February 1952,) and 250 ml. of 100 ppm. MCPA solution, prepared from the "active" perfusate drained from a MCPA enriched perfuser (Table 51b,) on 23/8/52. First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
0	57.1	96,	98,	102,	118,	25,	25,	28,	30,	3.5,	5,	55
3	49.7	75,	75,	81,	91,	32,	34,	34,	40,	4,	6,	45
9	73.6	52,	56,	65,	72,	23,	23,	26,	30,	2.5,	2.5,	60
15	59.6	35,	40,	40,	44,	23,	23,	30,	30,	3.5,	5,	50
21	51.8	66,	67,	67,	89,	25,	25,	33,	46,	6,	6,	35
24	61.3	95,	100,	101,	115,	83,	88,	93,	103,	28,	29,	4
27b	52.8	104,	110,	116,	127,	76,	81,	81,	83,	89,	93,	<1

Day 27. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

27a	52.8	57,	63,	63,	66,	23,	25,	25,	27,	4,	5.5,	50
30	59.8	70,	72,	75,	75,	28,	32,	33,	35,	5,	5,	45
33	54.6	68,	84,	86,	91,	38,	44,	44,	48,	11,	13,	25
36	64.8	94,	105,	107,	131,	109,	111,	113,	119,	72,	102,	<1
39b	64.8	89,	91,	91,	102,	85,	85,	94,	100,	85,	88,	<1

Day 39. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

39a	64.8	45,	46,	51,	72,	20,	22,	22,	25,	1.5,	1.5,	90
42	64.8	59,	63,	63,	65,	23,	25,	25,	25,	3,	3,	65
45	74.3	66,	70,	74,	75,	47,	48,	50,	51,	11,	14,	15
48b	64.8	83,	93,	103,	114,	91,	97,	103,	111,	100,	105,	<1

Day 48. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

48a	64.8	59,	63,	71,	75,	20,	25,	28,	31,	4,	5,	65
51	64.8	74,	79,	82,	82,	19,	21,	21,	34,	11,	13,	15
54	76.5	90,	99,	113,	113,	84,	93,	94,	119,	88,	97,	<1
57b	80.3	89,	90,	90,	99,	90,	96,	97,	107,	96,	117,	<1

Table 51. continued.

Day 57. Perfuser drained and refilled with 250 ml. of 5 ppm. 2-CPA solution. Perfuser re-started, sampled after 1 hr.

A.	B.	D. D. D. D.	E. E. E. E.	F. F.	H.
57a	78.3	87, 91, 92, 96,	52, 59, 59, 70,	23, 36,	5.5
77	65.0	97,105,108,111,	62, 65, 71, 85,	25, 26,	4.0
82	66.7	93, 96, 99, 99,	41, 42, 51, 62,	24, 25,	4.5
87	75.1	84, 87, 91, 98,	80, 83,101,101,	48, 49,	1.0
92	76.2	83, 96,106,114,	93, 94,113,114,	94,107,	<0.1
97b	63.7	107,107,113,115,	86, 94,105,110,	94,100,	<0.1

Day 97. Perfuser drained and refilled with 250 ml. of 5.0 ppm. 2-CPA solution. Perfuser re-started, sampled after 1 hr.

97a	63.7	50, 60, 61, 99,	36, 36, 38, 41,	22, 27,	4.0
102	45.3	75, 82, 84, 88,	40, 53, 64, 64,	24, 31,	4.0
107	81.6	79, 82, 86, 90,	38, 39, 39, 45,	23, 27,	5.0
112	75.4	100,103,113,117,	63, 76, 77, 81,	28, 43,	2.0
116	66.6	75, 75, 78, 80,	39, 42, 53, 60,	32, 41,	2.0
142	68.0	79, 85, 87, 90,	82, 84,103,106,	43, 51,	1.0
147	71.0	62, 62, 67, 89,	34, 41, 41, 45,	46, 51,	1.0
152	78.5	74, 80, 83, 95,	46, 47, 57, 78,	60, 62,	0.5
157	80.5	87, 91, 93, 93,	77, 81, 81, 88,	42, 69,	0.5

Table 51a. Transferred adaptation to MCPA, followed by 2-CPA.

Key to columns in table: as in Table 51, above.

Perfusion started on 17/8/52 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried February 1952,) and 250 ml. of 100 ppm. MCPA solution, prepared from the "active" perfusate drained from a MCPA enriched perfuser (Table 51b,) on 17/8/52. First sample taken after 1 hr. perfusion.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	G.
0	58.6	53, 55, 60, 62,	21, 24, 24, 24,	3.5,3.5,	80
3	67.3	62, 70, 74, 86,	18, 19, 21, 27,	3,4.5,	80
6	61.9	86, 86, 94,102,	28, 29, 31, 36,	5,6.5,	40
9	62.8	72, 72, 73, 92,	32, 33, 33, 35,	6.5, 8,	30
15	73.6	87, 90, 91, 91,	65, 67, 83,109,	42, 53,	20
18b	54.9	105,109,111,126,	80, 89, 91,109,	62, 69,	1

Day 18. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

18a	63.0	52, 54, 57, 70,	21, 21, 22, 25,	3, 3,	80

Table 51a. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
24	56.7	39,	44,	48,	53,	35,	39,	39,	42,	7,	7,	25
27	51.8	71,	75,	87,	89,	73,	81,	83,	122,	25,	31,	5
30b	61.3	77,	85,	87,	98,	108,	113,	123,	124,	62,	67,	1

Day 30. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

30a	52.0	83,	87,	87,	88,	25,	27,	31,	44,	4,	6,	50
33	52.8	64,	66,	70,	72,	36,	44,	49,	53,	5.5,	5.5,	35
36	59.8	90,	93,	93,	107,	92,	95,	98,	107,	90,	118,	<1
39b	55.1	93,	114,	118,	171,	98,	98,	100,	127,	91,	96,	<1

Day 39. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

39a	55.1	42,	47,	51,	53,	20,	22,	22,	24,	3.5,	3.5,	55
42	55.1	67,	74,	85,	94,	24,	25,	31,	34,	3.5,	5.5,	45
45b	55.1	73,	76,	76,	83,	74,	83,	87,	94,	71,	74,	1

Day 45. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

45a	55.1	58,	67,	74,	78,	22,	24,	24,	25,	3.5,	3.5,	60
48	55.1	67,	67,	83,	93,	29,	32,	32,	32,	2,	5.5,	35
51	74.3	92,	93,	94,	96,	86,	90,	105,	111,	81,	86,	<1

Day 54. Perfuser drained and refilled with 250 ml. of 10 ppm. 2-CPA solution. Perfuser re-started, sampled after 1 hr.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	H.
54a	64.8	83,	86,	88,	109,	42,	43,	46,	59,	20,	22,	10.0
59	66.4	78,	86,	93,	98,	32,	35,	38,	47,	26,	27,	6.0
64	81.3	99,	101,	107,	111,	70,	71,	73,	76,	25,	28,	2.5
69	77.5	99,	102,	103,	112,	71,	71,	75,	84,	30,	39,	2.0
74	60.3	88,	88,	88,	105,	56,	60,	60,	63,	35,	37,	2.0
79	75.9	82,	88,	91,	93,	82,	87,	101,	109,	55,	56,	0.6
84	65.0	101,	105,	108,	115,	82,	88,	92,	97,	40,	48,	0.8
89	69.8	98,	103,	109,	113,	75,	76,	76,	96,	40,	40,	1.5
94	81.2	71,	75,	81,	85,	80,	80,	81,	99,	92,	97	<0.1
99	70.5	101,	101,	105,	107,	112,	112,	113,	132,	109,	113,	<0.1
104b	66.0	94,	100,	103,	108,	61,	68,	70,	74,	58,	67,	0.4

Day 104. Perfuser drained and refilled with 250 ml. of 10 ppm. 2-CPA solution. Perfuser re-started, sampled after 1 hr.

104a	66.0	73,	77,	82,	85,	32,	36,	39,	39,	18,	18,	12.0
114	79.7	95,	98,	99,	107,	58,	64,	71,	73,	24,	29,	4.0
119	65.0	89,	91,	94,	100,	60,	66,	71,	72,	28,	34,	3.0
122	66.6	93,	95,	102,	104,	59,	60,	62,	72,	30,	33,	2.5
144	66.6	98,	98,	101,	102,	50,	60,	65,	87,	30,	41,	2.0

Table 51a. continued.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	H.
149	65.3	89,	92,	103,	115,	72,	73,	83,	84,	52,	52,	0.8
154	67.8	81,	84,	85,	100,	43,	46,	56,	59,	63,	71,	0.5
159	81.3	91,	92,	95,	99,	73,	79,	90,	93,	36,	37,	2.0
164	84.3	90,	95,	97,	107,	74,	77,	84,	102,	62,	65,	0.4

Table 51b. Transferred adaptation to MCPA, followed by 2-CPA.

Key to columns in table: as in Table 51, above.

Perfusion started on 9/6/52 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried February 1952,) and 250 ml. of 100 ppm. MCPA solution, prepared from the "active" perfusate drained from a MCPA enriched perfuser (Table 61a,) on 9/6/52. First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
0	48.9	61,	65,	94,	102,	31,	33,	33,	35,	4,	4,	55
3	48.0	79,	92,	98,	121,	29,	31,	33,	38,	6,	6,	30
6	46.5	71,	82,	88,	105,	17,	24,	32,	58,	4.5,	6.5,	45
9	44.1	86,	95,	100,	118,	39,	41,	45,	54,	4.5,	4.5,	25
12	52.5	74,	78,	84,	93,	29,	29,	38,	61,	4,	9.5,	30
15	57.7	69,	90,	95,	116,	43,	45,	45,	71,	5,	5,	25
18	46.3	82,	91,	93,	97,	56,	60,	60,	65,	4.5,	6.5,	15
21	40.7	84,	89,	96,	109,	44,	54,	69,	86,	7.5,	12,	15
24	47.0	94,	106,	128,	147,	77,	100,	100,	134,	23,	26,	5
27	52.5	84,	90,	95,	103,	55,	63,	86,	91,	72,	80,	<1

Day 30. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

30a	54.0	57,	70,	82,	96,	30,	31,	31,	33,	5.5,	7.5,	40
33	44.8	85,	103,	105,	110,	51,	51,	54,	54,	6.5,	11,	20
36	52.1	111,	111,	132,	144,	81,	81,	88,	90,	23,	27,	5
39	35.5	62,	68,	76,	86,	90,	90,	104,	147,	102,	124,	<1

Day 42. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

42a	46.8	94,	103,	103,	113,	32,	41,	41,	47,	6.5,	6.5,	30
45	47.3	87,	89,	93,	95,	32,	36,	42,	44,	10,	15,	30
48	61.9	94,	95,	102,	105,	89,	92,	95,	115,	21,	21,	5
60b	62.8	94,	102,	105,	115,	91,	94,	104,	113,	85,	86,	<1

Table 51b, continued.

Day 60. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	G.
60a	67.5	51, 58, 59, 80,	24, 25, 27, 31,	6,7.5,	90
63	67.5	68, 71, 89, 93,	25, 28, 28, 46,	6, 6,	45
66	57.2	87, 87, 96,101,	80, 94, 96,105,	35, 42,	2.5
69b	58.6	78, 78, 87, 97,	77, 82,101,102,	80,102,	<1

Day 69. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

69a	58.6	50, 51, 53, 58,	22, 22, 24, 26,	1.5,3.5,	80
72	67.3	86, 94,101,109,	70, 71, 73, 85,	19, 20,	10
75b	57.1	116,118,123,137,	91,100,102,116,	96,110,	<1

Day 75. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

75a	57.1	91, 93, 96,107,	30, 33, 37, 37,	3.5, 7,	30
78	62.8	99, 99, 99,108,	91, 92, 97, 99,	80, 99,	<1
81b	86.2	101,101,102,112,	91, 93,102,102,	95, 99,	<1

Day 81. Perfuser drained and refilled with 250 ml. of 10 ppm. 2-CPA solution. Perfuser re-started, sampled after 1 hr.

A.	B.	D. D. D. D.	E. E. E. E.	F. F.	H.
81a	60.0	78, 85, 95, 98,	40, 42, 50, 52,	18, 22,	11.0
86	72.0	78, 82, 83,101,	24, 25, 26, 40,	24, 25,	7.0
91	66.9	52, 61, 64, 66,	49, 51, 51, 54,	25, 25,	4.0
96	51.8	62, 68, 75, 79,	37, 46, 48, 56,	23, 25,	5.0
106	54.6	93, 95,106,117,	93,103,105,114,	33, 35,	2.5
111	54.6	70, 75, 75, 79,	82, 82, 88, 92,	37, 38,	1.5
116	63.5	91, 96,102,105,	66, 88, 93, 95,	33, 36,	2.0
126	64.8	46, 49, 52, 52,	49, 49, 51, 74,	28, 34,	2.5
131	78.3	95,100,102,117,	90, 95,106,109,	59, 60,	0.5
136	75.0	80, 84, 93,100,	96, 96,101,109,	92, 97,	<0.1
140b	77.5	92, 94,101,103,	72, 79, 81, 88,	53, 66,	0.5

Day 140. Perfuser drained and refilled with 250 ml. of 10 ppm. 2-CPA solution. Perfuser re-started, sampled after 1 hr.

140a	65.4	96, 98, 98,115,	47, 49, 55, 64,	21, 23,	5.5
145	72.2	94,100,100,114,	40, 51, 53, 68,	21, 26,	5.0
150	50.0	104,110,118,136,	50, 52, 58, 66,	20, 24,	5.5
155	68.8	96, 96, 96,121,	54, 55, 60, 60,	22, 26,	5.0
160	73.1	74, 77, 77, 86,	49, 52, 56, 66,	30, 37,	5.0
165	66.5	84, 89, 96, 99,	71, 75, 78, 81,	27, 41,	2.5

Table 51b.continued.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	H.
170	83.0	98,	100,	101,	110,	71,	75,	81,	93,	36,	37,	2.0
175	58.3	86,	91,	93,	93,	100,	100,	103,	105,	67,	67,	0.25
180	62.9	78,	83,	87,	103,	78,	87,	89,	99,	52,	60,	0.5
185	85.1	86,	87,	93,	95,	83,	83,	85,	95,	81,	86,	0.1

Day 190. Perfuser drained and refilled with 250 ml. of 10 ppm. 2-CPA solution. Perfuser re-started, sampled after 1 hr.

190a	65.0	88,	92,	95,	100,	55,	55,	60,	63,	23,	25,	6.5
215	73.3	79,	79,	84,	87,	48,	48,	48,	53,	19,	25,	9.0
220	78.1	68,	69,	82,	83,	38,	42,	44,	44,	27,	32,	11.0
225	76.0	75,	79,	83,	96,	47,	49,	57,	59,	24,	25,	7.0
230	73.5	79,	85,	93,	99,	52,	53,	56,	64,	38,	41,	6.5
235	76.6	53,	65,	77,	83,	44,	46,	53,	57,	17,	18,	8.0
240	87.1	86,	86,	93,	99,	55,	58,	61,	69,	29,	30,	5.5

Table 52. Transferred adaptation to MCPA, followed by 3-CPA.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Longest roots at nominal concentration of 10 ppm. as % m.c.
G. Indicated MCPA concentration in the perfusate (ppm.).
H. Indicated 3-CPA concentration in the perfusate (ppm.).

Perfusion started on 19/4/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 100 ppm. MCPA solution prepared from the combined "active" perfusates drained from two MCPA enriched perfusers (Tables 59 and 60 b,) on 19/4/53. Solution common with that in Table 58a. First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
0	74.9	56,	65,	71,	76,	16,	17,	19,	32,	1.5,	1.5,	90
6	70.7	48,	55,	56,	61,	14,	14,	16,	19,	1.5,	3,	105
12	81.4	51,	53,	55,	75,	17,	17,	18,	19,	1,	2.5,	85
18	80.6	51,	52,	52,	56,	15,	15,	16,	16,	2.5,	2.5,	95
24	80.7	92,	94,	94,	109,	33,	33,	38,	40,	6,	8.5,	40
30	87.6	81,	82,	93,	103,	85,	87,	105,	107,	96,	105,	<1

Day 33. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

33a	88.3	71,	71,	82,	89,	13,	14,	15,	23,	2.5,	2.5,	110
36	89.8	56,	58,	64,	69,	14,	16,	17,	19,	2,	2,	100
39	95.0	79,	82,	84,	88,	28,	28,	28,	28,	3,	5,	50
42	86.9	95,	96,	96,	97,	97,	102,	107,	107,	94,	102,	<1

Day 45. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

45a	100.4	66,	69,	70,	73,	14,	14,	18,	21,	2,	3,	105
48	95.7	88,	92,	92,	98,	31,	32,	34,	37,	4,	6.5,	40
51	100.5	98,	100,	104,	116,	104,	105,	106,	109,	103,	105,	<1

Day 54. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

54a	91.9	73,	74,	82,	92,	16,	18,	19,	21,	1,	2,	80
57	91.7	96,	97,	101,	112,	41,	45,	53,	60,	12,	13,	20
60b	93.7	94,	95,	98,	101,	82,	89,	94,	101,	87,	94,	1

Day 60. Perfuser drained and refilled with 250 ml. of 10 ppm. 3-CPA solution. Perfuser re-started, sampled after 1 hr.

Table 52. continued.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	H.
60a	104.1	74,	77,	83,	92,	37,	38,	39,	40,	14,	23,	12.0
63	104.1	87,	89,	89,	92,	94,	99,	100,	110,	59,	64,	0.4
66b	98.1	100,	106,	107,	109,	97,	97,	99,	103,	77,	96,	0.1

Day 66. Perfuser drained and refilled with 250 ml. of 10 ppm. 3-CPA solution. Perfuser re-started, sampled after 1 hr.

66a	98.1	63,	63,	66,	66,	39,	43,	44,	47,	18,	20,	12.0
69	99.8	87,	89,	92,	105,	77,	83,	88,	92,	50,	51,	1.0
72	102.7	93,	93,	102,	103,	90,	102,	103,	105,	74,	78,	0.1

Day 75. Perfuser drained and refilled with 250 ml. of 100 ppm. 3-CPA solution. Perfuser re-started, sampled after 1 hr.

75a	99.3	75,	80,	86,	97,	48,	49,	51,	61,	18,	18,	90
78	98.6	76,	77,	83,	91,	44,	45,	50,	53,	27,	30,	80
81	95.2	98,	99,	103,	112,	97,	98,	103,	104,	99,	105,	<1

Day 84. Perfuser drained and refilled with 250 ml. of 100 ppm. 3-CPA solution. Perfuser re-started, sampled after 1 hr.

84a	102.9	79,	79,	85,	88,	49,	50,	53,	55,	17,	21,	100
87	99.6	93,	97,	99,	107,	69,	72,	72,	73,	38,	42,	20
90	105.1	91,	92,	95,	100,	99,	99,	103,	107,	95,	99,	<1

Table 53. Transferred adaptation to MCPA, followed by 4-CPA.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Indicated MCPA concentration in the perfusate (ppm.).
G. Indicated 4-CPA concentration in the perfusate (ppm.).

Perfusion started on 30/8/52 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried February 1952,) and 250 ml. of 100 ppm. MCPA solution, prepared from the "active" perfusate drained from a MCPA enriched perfuser (Table 51b,) on 29/8/52. First sample taken after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	59.3	78,	79,	81,	88,	14,	14,	16,	16,	5,	7,	80
6	57.9	59,	66,	73,	83,	19,	19,	22,	24,	3.5,	3.5,	75
12	61.2	49,	56,	57,	60,	23,	26,	33,	36,	3.5,	3.5,	50
18	52.0	61,	67,	69,	75,	25,	25,	25,	27,	6,	7.5,	50
21	61.4	82,	85,	85,	91,	39,	46,	46,	49,	6.5,	8,	25
27b	63.5	93,	95,	99,	102,	91,	91,	102,	109,	80,	87,	1

Day 27. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

27a	63.5	66,	72,	89,	89,	19,	20,	20,	20,	1.5,	3,	75
30	51.2	74,	80,	82,	90,	19,	22,	23,	33,	4,	6,	70
33	49.0	73,	75,	80,	88,	31,	35,	37,	42,	6,	10,	35
36	63.5	69,	74,	76,	93,	54,	63,	65,	69,	16,	19,	10
39b	61.6	72,	80,	94,	102,	91,	107,	109,	114,	101,	106,	1

Day 39. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

39a	61.6	44,	45,	45,	49,	18,	19,	20,	21,	1.5,	3,	70
42	66.4	65,	68,	68,	74,	21,	21,	23,	26,	4.5,	4.5,	65
45	66.4	84,	90,	90,	92,	85,	89,	93,	99,	69,	104,	1
48b	75.0	89,	91,	97,	111,	104,	104,	112,	112,	97,	109,	1

Day 48. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
48a	75.0	83,	83,	88,	92,	24,	24,	28,	31,	6.5,	11,	100
50	80.3	87,	94,	99,	101,	47,	52,	59,	66,	7.5,	15,	35
52	78.3	86,	90,	111,	117,	67,	67,	77,	99,	22,	23,	10
54	75.0	92,	93,	95,	116,	109,	111,	127,	140,	96,	101,	1
56b	65.4	100,	101,	104,	110,	98,	107,	107,	112,	84,	100,	1

Table 53. continued.

Day 56. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	G.
56a	77.5	71, 73, 76, 84,	26, 28, 31, 31,	4,6.5,	90
58	77.5	62, 65, 78, 96,	32, 35, 35, 39,	6.5, 8,	35
60	65.4	84, 92,107,109,	78, 86, 89,115,	100,106,	1
62b	68.6	99,102,108,115,	99,105,107,112,	80, 93,	1

Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr. Day 62.

62a	68.6	80, 85, 88, 95,	22, 25, 26, 26,	4.5,4.5,	100
64	67.8	66, 69, 78, 91,	35, 38, 38, 43,	4.5, 9,	50
66	65.9	99,103,111,117,	97,103,120,122,	97,108,	1
68b	50.0	100,102,106,112,	126,148,152,164,	88, 88,	1

Day 68. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
68a	50.0	66, 74, 76, 78,	20, 22, 22, 24,	2, 2,	75
70	65.0	65, 69, 74, 75,	17, 23, 25, 26,	4.5,4.5,	50
72	60.6	86, 94,117,121,	45, 48, 50, 73,	13, 15,	15
75	64.7	90, 90, 91,107,	96, 99,100,122,	83,128,	1
77b	69.8	92, 95, 95, 98,	95, 98, 99,107,	99,100,	1

Day 77. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started sampled after 1 hr.

77a	74.4	55, 57, 62, 90,	13, 17, 18, 22,	2.5,2.5,	85
79	59.7	57, 69, 72, 84,	22, 22, 23, 27,	3.5,3.5,	65
81	75.1	95, 97, 98,104,	89, 93, 97, 97,	92, 96,	1
83	72.5	101,103,108,128,	96, 98,101,103,	103,103,	1

Table 54. Direct perfusion of MCPA / 4-CPA mixture.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. (relative to MCPA component,) as percentage mean control.
D. Longest roots at nominal dilution concentration of 0.1 ppm. (relative to MCPA component,) as percentage mean control.
E. Longest roots at nominal dilution concentration of 1.0 ppm. (relative to MCPA component,) as percentage mean control.
F. Indicated total activity of the perfusate as ppm. MCPA.

Perfusion started on 1/6/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of solution containing 100 ppm. MCPA and 1.0 ppm. 4-CPA. Sampled after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	100.4	66;	67,	68,	78,	13,	13,	15,	16,	1,	2,	100
9	88.7	62,	69,	72,	73,	12,	13,	14,	15,	2.5,	2.5,	100
12	88.3	64,	64,	65,	73,	16,	17,	17,	21,	1,	2,	80
15	94.9	65,	70,	74,	80,	12,	13,	15,	16,	1,	1,	100
18	94.4	72,	74,	76,	95,	21,	23,	26,	30,	2,	3,	45
24	100.1	74,	76,	77,	79,	15,	15,	16,	17,	1,	2,	90
30	101.4	75,	77,	80,	85,	17,	18,	19,	20,	3,	3,	50
36	96.6	86,	89,	91,	106,	22,	23,	25,	27,	3,	3,	50

Table 54a. Direct perfusion of MCPA / 4-CPA mixture.

Key to columns in table: as in Table 54, above.

Details of perfusion set-up: as in Table 54, above, except that the solution contained 100 ppm. MCPA and 10 ppm. 4-CPA.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	100.4	46,	47,	47,	50,	12,	12,	13,	14,	2,	2,	120
9	88.7	61,	65,	70,	71,	14,	19,	21,	26,	2,	2,	100
12	88.3	52,	61,	64,	65,	12,	14,	16,	17,	2,	2,	100
15	94.9	64,	68,	73,	74,	16,	16,	16,	16,	1,	2,	90
18	94.4	64,	68,	70,	78,	21,	22,	24,	28,	2,	3,	90
24	100.1	81,	81,	82,	90,	18,	19,	21,	22,	2,	3,	70
30	101.4	64,	69,	69,	74,	15,	15,	16,	18,	2,	3,	95
36	96.6	81,	88,	90,	92,	24,	24,	25,	26,	2,	3,	50
42	102.9	83,	85,	85,	88,	56,	59,	63,	75,	10,	18,	12
48b	105.1	95,	99,	103,	106,	100,	100,	102,	108,	95,	102,	<1

Day 48. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

Table 54a. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
48a	105.1	87,	88,	88,	96,	20,	21,	21,	26,	3,	3,	60
51	102.9	82,	84,	89,	93,	22.	25,	25,	26,	2,	3,	50
54	100.1	78,	83,	84,	89,	25,	27,	31,	33,	3,	3,	40
57	101.2	90,	90,	92,	96,	31,	32,	36,	41,	3,	4,	35
60	99.4	91,	92,	94,	94,	48,	48,	50,	53,	6,	7,	25

Day 64. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

64a	102.3	70,	70,	74,	78,	19,	23,	24,	30,	2,	3,	65
69	96.4	71,	77,	86,	86,	18,	19,	21,	25,	3,	4,	65
75	96.4	79,	85,	86,	86,	23,	23,	27,	29,	3,	4,	60
81	100.6	91,	94,	94,	97,	27,	29,	30,	31,	4,	5,	45

Table 54b. Direct perfusion of MCPA / 4-CPA mixture.

Key to columns in table: as in Table 54, above.

Details of perfusion set-up: as in Table 54, above, except that the solution contained 100 ppm. MCPA and 100 ppm. 4-CPA.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	100.4	58,	59,	66,	78,	9,	9,	10,	11,	1,	2,	190
9	88.7	42,	43,	44,	47,	10,	11,	12,	13,	1,	1,	170
12	88.3	54,	55,	63,	76,	10,	11,	15,	17,	1,	1,	140
15	94.9	50,	50,	55,	57,	9,	10,	11,	13,	1,	2,	150
18	94.4	81,	82,	86,	87,	23,	24,	24,	24,	3,	3,	55
24	100.1	74,	78,	85,	96,	23,	26,	28,	28,	4,	6,	45
30	101.4	77,	78,	79,	82,	24,	28,	29,	32,	5,	6,	40
36	96.6	93,	96,	101,	101,	34,	35,	36,	37,	5,	6,	35
42	102.9	83,	89,	101,	116,	57,	62,	67,	71,	8,	12,	13
48b	105.1	99,	103,	105,	111,	79,	84,	84,	86,	19,	22,	8

Day 48. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

48a	105.1	87,	88,	92,	94,	16,	18,	23,	24,	2,	3,	65
51	102.9	85,	88,	89,	91,	22,	23,	26,	30,	3,	3,	50
54	100.1	90,	90,	96,	104,	36,	36,	40,	41,	4,	4,	30
57	101.2	91,	92,	92,	98,	59,	59½	61,	63,	10,	15,	12
60	99.4	91,	93,	104,	107,	93,	95,	98,	103,	88,	91	<1

Table 54b. continued.

Day 64. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
64a	102.3	70,	70,	74,	78,	19,	23,	24,	30,	2,	3,	65
69	96.4	81,	83,	90,	97,	24,	24,	27,	30,	3,	4,	55
75	96.4	87,	89,	89,	95,	29,	31,	34,	34,	4,	6,	35
81	100.6	92,	95,	96,	100	43,	44,	45,	52,	6,	7,	25
91	99.1	93,	96,	100,	102;	81,	82,	83,	86,	13,	17,	10

Table 55.

Direct perfusion of MCPA in mixture with 2,4-D and 4-CPA.

Key to columns in table:

A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm, (relative to the MCPA,) as percentage mean control.
D. Longest roots at nominal dilution concentration of 0.1 ppm. (relative to the MCPA,) as percentage mean control.
E. Longest roots at nominal dilution concentration of 1.0 ppm. (relative to the MCPA,) as percentage mean control.
F. Indicated total activity in the perfusate, as ppm. MCPA.

Perfusion started on 20/7/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of solution containing 100 ppm. MCPA, 10 ppm. 2,4-D and 10 ppm. 4-CPA. Solution sampled before adding to the perfuser.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	98.4	83,	84,	85,	87,	17,	17,	18,	19,	2,	3,	85
6	99.8	86,	87,	89,	92,	17,	18,	21,	24,	3,	4,	65
12	98.4	75,	79,	86,	89,	17,	17,	18,	23,	2,	3,	85
18	93.8	74,	75,	77,	85,	16,	16,	17,	18,	2,	2,	90
21	93.9	69,	72,	74,	79,	20,	20,	21,	25,	2,	3,	100
24	97.5	67,	70,	80,	84,	13,	14,	15,	16,	1,	2,	110
27	100.0	77,	78,	83,	98,	15,	15,	16,	17,	2,	3,	100
30	102.7	79,	84,	90,	91,	15,	18,	19,	20,	3,	3,	80
33	105.1	77,	79,	80,	83,	14,	15,	15,	17,	2,	2,	100
39	96.1	75,	77,	78,	81,	22,	22,	23,	25,	3,	4,	65
42	99.1	83,	83,	88,	96,	33,	35,	35,	36,	4,	5,	45

Table 55a. Direct perfusion of MCPA in mixture with 2,4-D.

Key to columns in table: as in Table 55, above.

Details of perfusion set-up, as for Table 55, above, except that the solution contained 100 ppm. MCPA and 10 ppm. 2,4-D only. Started on 20/7/53. Solution sampled before adding to perfuser.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	98.4	71,	78,	78,	80,	24,	27,	28,	30,	3,	3,	75
6	99.8	76,	77,	80,	96,	16,	17,	17,	20,	3,	3,	80
12	98.4	68,	68,	71,	71,	14,	14,	18,	19,	2,	3,	90
18	93.8	74,	74,	80,	87,	16,	16,	17,	18,	2,	2,	90
21	93.9	76,	78,	78,	82,	21,	21,	23,	25,	2,	3,	85

Table 55a. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
24	97.5	61,	62,	67,	79,	14,	16,	19,	23,	2,	2,	90
27	100.0	77,	78,	80,	82,	16,	16,	17,	20,	2,	2,	90
30	102.7	77,	80,	89,	98,	16,	17,	18,	19,	3,	3,	80
33	105.1	78,	78,	79,	84,	17,	17,	18,	19,	2,	3,	85
39	96.1	82,	82,	87,	89,	17,	20,	21,	26,	2,	3,	70
42	99.1	81,	84,	85,	91,	27,	30,	30,	37,	3,	4,	40

Table 55b. Direct perfusion of MCPA in mixture with 2,4-D.

Key to columns in table: as in Table 55, above.

Details of perfusion set-up, as for Table 55, above, except that the solution contained 100 ppm. MCPA and 100 ppm. 2,4-D only. Started on 20/7/53. Solution sampled before adding to perfuser.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	98.4	67,	68,	69,	75,	11,	12,	13,	15,	2,	2,	115
6	99.8	66,	70,	77,	82,	11,	11,	11,	13,	1,	2,	135
12	98.4	56,	59,	67,	69,	8,	10,	11,	14,	2,	2,	135
18	93.8	77,	77,	78,	82,	12,	13,	13,	16,	2,	2,	120
21	93.9	83,	87,	97,	102,	26,	27,	31,	35,	4,	5.5,	40
24	97.5	87,	88,	100,	101,	48,	49,	51,	63,	8,	19,	20
27	100.0	98,	99,	100,	104,	70,	71,	75,	78,	11,	16,	10
30b	96.3	102,	102,	106,	111,	87,	88,	92,	92,	30,	31,	4

Day 30. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

30a	96.3	84,	85,	86,	92,	24,	25,	28,	31,	3,	4,	45
33	105.1	81,	84,	84,	88,	21,	22,	24,	24,	3,	4,	60
36	100.6	83,	88,	89,	91,	25,	34,	35,	39,	4,	5,	35
39	96.1	87,	87,	89,	99,	28,	29,	29,	30,	5,	5,	40
42	99.1	81,	82,	84,	88,	28,	29,	29,	34,	4,	5,	40

Table 56. Transferred adaptation to MCPA, followed by 2,4-D.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Indicated MCPA concentration in the perfusate (ppm.).
G. Indicated 2,4-D concentration in the perfusate (ppm.).

Perfusion started on 5/2/52 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried June 1951,) and 250 ml. of 100 ppm. MCPA solution, prepared from the "active" perfusate drained from a MCPA enriched perfuser (Table 57d,) on 4/2/52. First sample taken after 1 hr perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	72.6	78,	82,	82,	87,	19,	19,	23,	25,	4,	4,	80
4b	68.4	92,	104,	107,	118,	47,	50,	61,	72,	17,	19,	9

Day 4. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

4a	68.4	56,	59,	63,	68,	17,	18,	19,	21,	3,	4.5,	85
7	60.9	63,	63,	64,	92,	21,	21,	25,	26,	3.5,	6.5,	75
9	75.2	49,	51,	67,	99,	20,	21,	24,	27,	2.5,	4,	65
11	57.0	79,	86,	102,	111,	44,	56,	63,	79,	3.5,	16,	20
13	83.8	79,	83,	87,	102,	62,	65,	78,	92,	95,	117,	1

Day 15. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution.Perfuser re-started, sampled after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
15a	69.5	66,	72,	75,	92,	17,	17,	18,	20,	4.5,	7,	130
18	64.1	80,	97,	102,	117,	80,	88,	103,	111,	84,	130,	1

Day 20. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

20a	63.7	66,	85,	86,	98,	17,	18,	21,	24,	3,	4.5,	110
22	58.1	60,	62,	97,	117,	74,	83,	86,	91,	21,	24,	10
24	55.1	73,	75,	102,	124,	116,	118,	127,	146,	94,	138,	1

Day 26. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 10 ppm. urethane. Perfuser re-started, sampled after 1 hr.

26a	69.3	70,	77,	80,	91,	16,	16,	16,	20,	3,	4.5,	110
28	61.2	62,	64,	77,	90,	26,	29,	31,	33,	3.5,	5,	70
30	71.1	100,	103,	111,	120,	90,	93,	101,	108,	114,	148,	1

Table 56. continued.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	G.
32	69.4	88,102,118,134,	88, 88, 90, 92,	95,105,	1

Day 33. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 100 ppm. urethane. Perfuser re-started, sampled after 1 hr.

33a	84.3	75, 78, 88, 90,	21, 21, 23, 26,	2.5,4.5,	100
35	71.9	95,116,119,127,	35, 45, 45, 53,	5.5, 7,	40
37	70.0	69, 70, 80, 91,	93, 96,106,123,	99,110,	1
39	65.3	88, 88, 94, 97,	80, 83,101,123,	90,100,	1

Day 40. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 1,000 ppm. urethane. Perfuser re-started, sampled after 1 hr.

40a	54.4	81, 96,103,116,	22, 24, 24, 26,	3.5, 11,	90
42	72.5	64, 65, 95, 99,	33, 33, 37, 47,	11, 17,	55
44	63.1	103,113,124,150,	92, 97,113,130,	113,149,	1

Table 57. Transferred adaptation to MCPA, followed by 2,4,5-T.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Longest roots at nominal concentration of 10 ppm. as % m.c.
G. Indicated MCPA concentration in the perfusate (ppm.).
H. Indicated 2,4,5-T concentration in the perfusate (ppm.).

Perfusion started on 9/12/51 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried June 1951,) and 250 ml. of 100 ppm. MCPA solution prepared from the"active" perfusate drained from a MCPA enriched perfuser (Table 57d,) on 9/12/51. First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
0	70.3	44,	54,	64,	66,	15,	16,	19,	22,	1.5,	3,	100
5	67.8	43,	50,	62,	96,	25,	27,	30,	31,	3,	4.5,	50
8	48.8	88,	90,	113,	115,	16,	20,	21,	25,	2,	2,	70
21	73.3	42,	47,	52,	57,	18,	19,	23,	33,	4,	4,	80
24	81.3	80,	80,	89,	91,	27,	27,	28,	28,	5,	10,	45
27	77.7	55,	59,	61,	65,	78,	83,	85,	89,	73,	97,	<1
30b	72.0	75,	86,	93,	95,	84,	85,	95,	104,	57,	60,	1.5

Day 30. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

30a	72.0	67,	75,	78,	85,	19,	19,	21,	24,	3,	3,	70
33	59.0	70,	71,	75,	85,	24,	25,	27,	32,	3.5,	3.5,	60
36	59.7	77,	77,	82,	92,	18,	19,	22,	22,	3.5,	10,	70
38	59.1	83,	86,	95,	105,	29,	29,	30,	32,	5,	7,	40
40	66.3	50,	50,	51,	53,	23,	26,	33,	33,	9,	11,	20
42	51.2	78,	78,	80,	113,	47,	55,	57,	66,	14,	16,	10
44	43.2	79,	95,	109,	118,	63,	65,	72,	72,	23,	23,	5
46	49.9	84,	86,	94,	132,	102,	102,	114,	124,	76,	96,	<1
48b	58.4	88,	93,	99,	105,	74,	101,	106,	110,	129,	136,	<1

Day 48. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

48a	58.4	55,	62,	64,	65,	21,	22,	22,	24,	3.5,	5,	75
50	74.6	56,	57,	59,	92,	15,	17,	19,	21,	4,	5.5,	90
52	86.6	47,	53,	59,	66,	16,	19,	19,	22,	2.5,	2.5,	70
54	67.0	55,	58,	60,	82,	27,	28,	30,	33,	4.5,	7.5,	35
56	57.1	79,	79,	84,	86,	44,	47,	53,	60,	7,	16,	20
58	72.6	55,	64,	66,	75,	57,	59,	59,	61,	36,	39,	5
60	61.4	88,	91,	98,	122,	78,	102,	102,	106,	80,	83,	<1
62b	68.4	73,	86,	91,	107,	54,	78,	86,	92,	59,	66,	<1

Table 57. continued.

Day 62. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
62a	68.4	48,	57,	66,	104,	17,	19,	19,	22,	3,	4.5,	70
64	67.3	46,	54,	68,	76,	18,	19,	19,	20,	3,	4.5,	65
66	87.3	40,	41,	72,	80,	18,	20,	21,	28,	3.5,	4.5,	55
68	66.4	52,	55,	66,	105,	96,	99,	99,	106,	72,	87,	<1
70	86.7	83,	92,	97,	108,	89,	90,	90,	96,	79,	87,	<1
72	70.1	76,	87,	101,	117,	91,	94,	107,	114,	93,	96,	<1

Day 73. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4,5-T solution. Perfuser re-started, sampled after 1 hr.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	H.
73	75.2	64,	67,	69,	77,	22,	23,	23,	39,	2.5,	2.5,	90
78	62.3	69,	95,	97,	105,	17,	18,	19,	19,	3,	3,	90
83	53.4	51,	51,	54,	66,	7,	9,	11,	17,	2,	4,	100
88	71.1	99,	103,	104,	106,	11,	11,	17,	20,	3,	4,	90
93	69.9	67,	69,	79,	80,	17,	18,	23,	34,	3,	4.5,	90
98	54.4	64,	83,	99,	123,	22,	26,	28,	33,	3.5,	5.5,	55

Table 57a. Transferred adaptation to MCPA, followed by 2,4,5-T.

Key to columns in table: as in Table 57, above.

Perfusion started on 8/8/52 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried February 1952,) and 250 ml. of 100 ppm. MCPA solution, prepared from the perfusate drained from a MCPA enriched perfuser (Table 51b,) on 8/8/52. First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
0	61.4	64,	64,	65,	88,	23,	24,	24,	29,	5,	5,	50
3	61.4	73,	80,	87,	91,	24,	28,	28,	33,	3.5,	5,	45
6	57.2	67,	72,	86,	86,	24,	24,	24,	26,	3.5,	5,	50
9	58.6	73,	73,	75,	94,	26,	29,	29,	32,	3.5,	7,	40
12	67.3	55,	56,	63,	73,	22,	22,	24,	24,	3,	4.5,	55
15	61.9	81,	82,	87,	89,	23,	24,	31,	34,	3,	5,	50
18	62.8	80,	81,	84,	96,	29,	33,	37,	41,	9.5,	19,	30
21	86.2	81,	99,	100,	108,	105,	107,	110,	118,	43,	45,	2.5
24b	73.6	82,	82,	90,	120,	86,	91,	92,	117,	38,	42,	2.5

Day 24. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

Table 57a. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
24a	71.9	60,	68,	88,	88,	24,	25,	26,	29,	4,	4,	50
27	54.9	55,	57,	58,	73,	31,	33,	35,	38,	9,	15,	30
30	59.6	72,	89,	92,	107,	87,	92,	97,	106,	25,	32,	4.5
33b	61.2	77,	80,	83,	98,	100,	103,	114,	123,	56,	64,	1.5

Day 33. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

33a	61.2	39,	46,	49,	59,	23,	29,	29,	33,	5,	5,	40
36	51.8	71,	73,	75,	100,	37,	46,	48,	74,	13,	16,	10
39	61.3	92,	93,	97,	103,	100,	101,	103,	129,	72,	75,	1
42b	52.8	106,	114,	121,	131,	114,	114,	119,	119,	118,	136,	<1

Day 42. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

42a	52.8	64,	67,	74,	78,	28,	30,	30,	30,	2,	4,	40
45	59.8	70,	72,	75,	80,	27,	28,	28,	35,	5,	6.5,	40
51	67.6	86,	86,	98,	102,	93,	98,	114,	115,	93,	107,	<1
54b	67.6	117,	126,	126,	129,	107,	109,	111,	112,	86,	99,	<1

Day 54. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

54a	67.6	49,	50,	50,	58,	22,	24,	24,	30,	1.5,	3,	50
57	67.6	80,	99,	104,	107,	78,	87,	99,	108,	68,	114,	<1
60	74.3	81,	82,	84,	92,	106,	108,	120,	123,	85,	88,	<1

Day 63. Perfuser drained and refilled with 250 ml. of 10 ppm. 2,4,5-T solution. Perfuser re-started, sampled after 1 hr.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	H.
63a	64.8	66,	69,	77,	82,	22,	25,	26,	26,	1.5,	3,	6.5
68	66.4	82,	85,	98,	109,	92,	100,	100,	121,	58,	68,	0.1
73	81.3	89,	92,	116,	121,	94,	97,	105,	106,	97,	112,	<0.1
77b	77.5	88,	95,	98,	125,	94,	97,	102,	105,	90,	103,	<0.1

Day 77. Perfuser drained and refilled with 250 ml. of 1.0 ppm. 2,4,5-T solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	H.
77a	77.5	75,	77,	77,	86,	77,	80,	80,	89,	14,	23,	0.95
80	77.5	92,	95,	98,	105,	83,	88,	95,	98,	88,	97,	<0.1
83b	60.3	103,	105,	106,	108,	90,	98,	101,	136,	65,	88,	<0.1

Day 83. Perfuser drained and refilled with 250 ml. of 10 ppm. 2,4,5-T solution. Perfuser re-started, sampled after 1 hr.

Table 57a. continued.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	H.
83a	72.2	61,	65,	80,	84,	12,	14,	15,	15,	1.5,	3,	12.0
86	67.8	75,	75,	78,	80,	17,	19,	19,	19,	3,	4.5,	9.0
89	66.0	83,	88,	92,	115,	32,	32,	33,	47,	4.5,	6,	4.0
92	65.0	92,	98,	103,	106,	40,	40,	45,	57,	15,	22,	0.9
95	68.8	93,	105,	109,	112,	58,	61,	61,	79,	35,	41,	0.3
98	69.8	90,	96,	98,	100,	83,	85,	85,	93,	67,	70,	0.1
101b	73.1	86,	88,	97,	99,	82,	83,	93,	94,	86,	92,	<0.1

Day 101. Perfuser drained and refilled with 250 ml. of 50 ppm. 2,4,5-T solution. Perfuser re-started, sampled after 1 hr.

101a	73.1	19,	21,	22,	25,	4,	4,	4,	7,	1.5,	2.5,	70
111	77.9	26,	26,	27,	33,	4,	5,	6.5,	7.5,	1.5,	2.5,	50
121	68.0	41,	47,	64,	69,	7.5,	9,	10,	12,	3,	3,	25
131	77.1	47,	49,	49,	54,	14,	15,	15,	16,	2.5,	4,	12
161	82.8	43,	43,	46,	58,	6,	7,	9.5,	9.5,	1,	1,	25
171	75.3	44,	49,	50,	53,	12,	13,	13,	16,	2.5,	2.5,	16
181	85.9	47,	50,	55,	63,	8,	9.5,	11,	14,	1,	1,	20

Table 57b. Transferred adaptation to MCPA, followed by 2,4,5-T.

Key to columns in table: as in Table 57, above.

Perfusion started on 21/7/52 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried February 1952,) and 250 ml. of 100 ppm. MCPA solution, prepared from the "active" perfusate drained from a MCPA enriched perfuser (Table 51b,) on 21/7/52. First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
0	46.8	71,	88,	96,	120,	34,	34,	41,	45,	4.5,	6.5,	40
3	47.3	74,	78,	80,	93,	30,	30,	34,	51,	4,	6.5,	40
18	62.8	91,	97,	102,	115,	29,	30,	33,	33,	5,	6.5,	40
21	67.5	68,	80,	80,	83,	24,	25,	27,	31,	1.5,	4.5,	45
24	57.2	72,	84,	87,	96,	31,	33,	35,	38,	5,	5,	35
27	58.6	68,	79,	82,	87,	27,	29,	32,	32,	5,	5,	40
30	67.3	64,	67,	73,	73,	22,	31,	34,	36,	7.5,	12,	30
33	61.9	78,	79,	82,	108,	79,	89,	91,	92,	32,	37,	4
35b	49.7	83,	87,	91,	97,	77,	79,	89,	99,	79,	87,	<1

Day 35. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

35a	62.8	73,	78,	81,	88,	27,	29,	29,	30,	3,	3,	65

Table 57b. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
39	86.2	85,	87,	89,	100,	38,	46,	48,	57,	6,	7,	20
42	73.6	78,	83,	94,	98,	69,	69,	72,	73,	15,	20,	8
45b	54.9	88,	89,	102,	104,	104,	106,	111,	119,	102,	110,	<1

Day 45. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

45a	63.0	62,	67,	71,	75,	24,	25,	27,	30,	3,	3,	65
48	59.6	50,	52,	64,	75,	23,	29,	35,	39,	6.5,	8.5,	40
51	56.7	90,	92,	97,	116,	102,	106,	106,	123,	74,	97,	<1
54b	40.1	85,	85,	90,	97,	65,	65,	75,	105,	100,	115,	<1

Day 54. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

54a	40.1	62,	67,	82,	90,	32,	37,	40,	45,	7.5,	7.5,	25
57	61.3	92,	93,	95,	100,	49,	51,	56,	60,	15,	18,	15
60b	61.4	101,	103,	105,	117,	85,	98,	103,	108,	73,	113,	<1

Day 60. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

60a	61.4	54,	56,	59,	83,	25,	26,	28,	29,	3.5,	5,	45
63	59.8	60,	62,	68,	78,	37,	37,	45,	53,	10,	12,	15
66b	49.0	102,	104,	131,	143,	110,	112,	129,	129,	65,	78,	1

Day 66. Perfuser drained and refilled with 250 ml. of 10 ppm. 2,4,5-T solution. Perfuser re-started, sampled after 1 hr.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	H.
71	54.6	82,	84,	84,	86,	22,	22,	24,	28,	3.5,	3.5,	7.5
76	74.3	74,	82,	84,	89,	36,	36,	62,	78,	28,	31,	0.75
81	77.6	75,	84,	88,	90,	89,	93,	95,	98,	36,	75,	0.15
86	67.1	97,	100,	104,	118,	75,	81,	91,	98,	86,	104,	<0.1
91b	75.0	119,	124,	125,	127,	88,	91,	92,	103,	68,	87,	<0.1

Day 91. Perfuser drained and refilled with 250 ml. of 10 ppm. 2,4,5-T solution. Perfuser re-started, sampled after 1 hr,

91a	75.0	77,	87,	103,	112,	16,	18,	19,	20,	1.5,	4,	9.5
96	77.5	74,	74,	75,	78,	35,	37,	44,	55,	6.5,	6.5,	4.0
101	60.3	103,	105,	105,	108,	71,	71,	86,	95,	63,	66,	0.1
106b	65.9	76,	88,	106,	117,	85,	87,	94,	100,	83,	103,	<0.1

Day 106. Perfuser drained and refilled with 250 ml. of 10 ppm. 2,4,5-T solution. Perfuser re-started, sampled after 1 hr.

106a	65.9	56,	62,	73,	73,	16,	17,	21,	23,	1.5,	1.5,	11.0
111	65.0	94,	100,	101,	105,	26,	28,	28,	42,	6,	7.5	5.0

Table 57b. continued,

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	H.
115	69.8	82,	82,	89,	100,	54,	64,	64,	99,	34,	40,	0.3
121	81.2	85,	96,	106,	106,	76,	80,	81,	90,	91,	95,	<0.1
126b	70.5	112,	112,	118,	129,	92,	96,	96,	104,	52,	69,	0.1

Day 126. Perfuser drained and refilled with 250 ml. of 50 ppm. 2,4,5-T solution. Perfuser re-started, sampled after 1 hr.

126a	79.2	26,	28,	32,	33,	4,	6,	6,	8,	1.5,	1.5,	50
146	65.0	35,	35,	43,	45,	6,	7.5,	9,	11,	1.5,	3,	35
176	65.3	63,	63,	70,	89,	15,	18,	24,	32,	3,	4.5,	9

Table 57c. Transferred adaptation to MCPA, followed by 2,4,5-T.

Key to columns in table: as in Table 57, above.

Perfusion started on 20/1/52 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried June 1951,) and 250 ml. of 100 ppm. MCPA solution, prepared from the "active" perfusate drained from a MCPA enriched perfuser (Table 57d,) on 20/1/52. First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
0	51.2	53,	60,	96,	98,	20,	22,	24,	31,	4,	4,	65
6	58.4	52,	60,	70,	72,	15,	19,	19,	21,	3.5,	5,	70
9	86.6	60,	60,	61,	66,	13,	13,	15,	15,	2.5,	3.5,	110
12	67.0	45,	46,	49,	64,	15,	16,	16,	19,	3,	3,	90
14	57.1	39,	60,	65,	65,	14,	17,	23,	25,	3.5,	5.5,	75
16	72.6	51,	51,	57,	79,	18,	18,	20,	22,	3,	5.5,	70
18	61.4	80,	82,	91,	99,	23,	23,	23,	24,	3.5,	3.5,	55
21	61.8	57,	58,	76,	94,	19,	21,	23,	28,	5,	5,	65
24	87.3	80,	92,	93,	94,	25,	25,	25,	26,	8,	12,	50
27	57.0	65,	78,	120,	122,	47,	58,	67,	84,	7,	25,	10
29	83.8	80,	83,	86,	95,	78,	84,	87,	110,	57,	108,	<1

Day 31. Perfuser drained and refilled with 80 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

31a	69.5	74,	81,	105,	107,	20,	25,	26,	26,	3,	7,	50
33	56.9	65,	83,	95,	106,	32,	33,	35,	46,	7,	11,	30
35	63.5	62,	66,	85,	118,	52,	65,	66,	66,	8,	9.5,	10
37	63.7	80,	88,	105,	110,	91,	97,	104,	107,	52,	74,	1
39b	55.1	85,	89,	91,	109,	75,	94,	114,	127,	78,	98,	<1

Day 39. Perfuser drained and refilled with 250 ml. of 10 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

Table 57c. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
39a	55.1	78,	80,	85,	100,	56,	65,	71,	82,	24,	24,	10.0
41	53.4	62,	62,	66,	88,	107,	112,	131,	135,	45,	51,	2.0
43	69.3	84,	90,	90,	91,	81,	87,	98,	100,	72,	113,	< 1
45	58.0	124,	126,	129,	156,	95,	116,	136,	142,	45,	69,	1.5
47b	69.4	68,	74,	84,	98,	93,	99,	106,	121,	82,	82,	< 1

Day 47. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

47a	71.1	51,	63,	65,	66,	14,	17,	20,	23,	3,	3,	80
49	67.5	70,	79,	101,	110,	13,	15,	15,	25,	3,	4.5,	70
51	71.9	67,	75,	81,	88,	28,	31,	32,	39,	3,	4,	40
53	70.0	79,	79,	96,	107,	43,	44,	50,	51,	4.5,	5.5,	25
55	65.3	94,	98,	100,	115,	60,	61,	69,	86,	77,	98,	<1
57	64.4	56,	72,	75,	81,	67,	67,	75,	83,	87,	104,	<1

Day 58. Perfuser drained and refilled with 250 ml. of 10 ppm. 2,4,5-T solution. Perfuser re-started, sampled after 1 hr.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	H.
58a	63.1	49,	54,	67,	78,	14,	16,	24,	27,	3,	5,	8.0
60	63.1	71,	82,	98,	102,	22,	24,	30,	32,	5,	5,	5.5
63	69.6	53,	56,	73,	100,	60,	64,	76,	97,	19,	19,	0.9
65	60.3	103,	103,	115,	117,	93,	97,	105,	112,	60,	72,	0.1
67	56.0	56,	59,	68,	74,	41,	41,	61,	102,	54,	66,	0.1
69	69.6	54,	62,	77,	79,	26,	30,	37,	112,	50,	50,	0.1

Day 70. Perfuser drained and refilled with 250 ml. of 10 ppm. 2,4,5-T solution. Perfuser re-started, sampled after 1 hr.

70a	68.3	63,	65,	65,	69,	12,	12,	13,	19,	1.5,	3,	10.0
72	60.9	58,	94,	102,	140,	16,	20,	21,	23,	1.5,	2.5,	8.0
74	66.6	59,	60,	68,	89,	27,	29,	33,	36,	1.5,	1.5,	4.0
77	77.6	35,	36,	40,	43,	21,	21,	25,	26,	2.5,	2.5,	7.0
80	77.6	54,	61,	74,	89,	28,	31,	35,	36,	2.5,	4,	4.0
100	69.3	80,	87,	88,	105,	61,	68,	85,	99,	61,	73,	0.1
104	79.1	76,	85,	89,	104,	37,	37,	38,	40,	32,	44,	0.3
109	62.5	85,	104,	106,	122,	58,	61,	74,	85,	48,	56,	0.2
114b	69.0	58,	61,	64,	81,	49,	57,	81,	102,	39,	41,	0.3

Day 114. Perfuser drained and refilled with 250 ml. of 10 ppm. 2,4,5-T solution. Perfuser re-started, sampled after 1 hr.

114a	69.0	47,	52,	67,	81,	11,	12,	15,	18,	4.5,	4.5,	11.0
119	54.4	70,	70,	79,	98,	41,	42,	63,	74,	9,	17,	2.0
124	67.0	72,	81,	88,	106,	49,	57,	60,	66,	31,	33,	0.4
129	52.3	82,	88,	96,	105,	94,	98,	107,	111,	94,	121,	<0.1

Day 134. Perfuser drained and refilled with 250 ml. of 10 ppm. 2,4,5-T solution. Perfuser re-started, sampled after 1 hr.

Table 57c. continued.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	H.
134a	54.5	60,	62,	64,	70,	24,	26,	28,	35,	5.5,	5.5,	5.5
139	46.3	119,	119,	124,	130,	74,	76,	78,	108,	13,	15,	1.0
149b	44.1	91,	113,	129,	188,	68,	82,	88,	129,	66,	75,	0.1
Day 149. Perfuser drained and refilled with 250 ml. of 10 ppm. 2,4,5-T solution. Perfuser re-started, sampled after 1 hr.												
149a	44.1	73,	79,	86,	93,	11,	13,	18,	23,	4.5,	7,	10.0
154	52.5	70,	74,	76,	118,	42,	42,	51,	51,	5.5,	14,	2.5
159	46.3	104,	114,	114,	117,	78,	84,	86,	99,	24,	41,	0.4
164b	41.9	91,	98,	105,	112,	81,	84,	117,	124,	55,	74,	0.1
Day 164. Perfuser drained and refilled with 250 ml. of 10 ppm. 2,4,5-T solution. Perfuser re-started, sampled after 1 hr.												
164a	41.9	79,	86,	91,	105,	29,	29,	36,	43,	5,	7,	5.0
169	55.2	100,	107,	112,	118,	36,	53,	54,	129,	51,	67,	0.15
174	44.8	71,	94,	105,	125,	78,	80,	100,	121,	83,	114,	<0.1
Day 179. Perfuser drained and refilled with 250 ml. of 20 ppm. 2,4,5-T solution. Perfuser re-started, sampled after 1 hr.												
179a	50.9	39,	41,	43,	75,	8,	12,	12,	14,	2,	4,	17.0
184	46.8	56,	83,	85,	122,	19,	21,	21,	26,	2,	6.5,	4.0
189	63.3	66,	73,	79,	84,	59,	66,	74,	79,	21,	21,	0.8
199	59.4	96,	101,	104,	115,	89,	104,	110,	113,	93,	103,	<0.1
Day 204. Perfuser drained and refilled with 250 ml. of 30 ppm. 2,4,5-T solution. Perfuser re-started, sampled after 1 hr.												
204a	67.5	27,	30,	33,	40,	7.5,	9,	10,	11,	0,	1.5,	32.0
209	61.9	31,	32,	37,	44,	6.5,	8,	8,	15,	1.5,	3,	33.0
214	53.0	47,	51,	51,	60,	7.5,	7.5,	9.5,	12,	2,	2,	23.0
219	62.8	49,	51,	54,	56,	9.5,	10,	13,	13,	1.5,	1.5,	16.0
224	68.8	96,	102,	108,	116,	36,	41,	41,	45,	6,	6,	2.5
229	57.9	71,	72,	93,	104,	21,	21,	21,	31,	36,	41,	0.3
234b	56.7	85,	86,	88,	109,	58,	62,	72,	106,	102,	104,	<0.1
Day 234. Perfuser drained and refilled with 250 ml. of 40 ppm. 2,4,5-T solution. Perfuser re-started, sampled after 1 hr.												
234a	61.2	23,	23,	25,	25,	6.5,	6.5,	6.5,	10,	1.5,	1.5,	45.0
239	49.2	37,	39,	41,	55,	8,	8,	12,	14,	0,	2,	30.0
244	61.4	28,	33,	34,	42,	6.5,	6.5,	8,	12,	0,	1.5,	40.0
254	54.6	33,	37,	40,	44,	3.5,	5.5,	5.5,	7.5,	2,	3.5,	45.0
259	74.3	27,	28,	34,	36,	6.5,	8,	9.5,	11,	0,	1.5,	30.0
264	77.6	39,	39,	39,	49,	6.5,	6.5,	7.5,	9,	1.5,	2.5,	35.0
269	67.1	48,	52,	54,	67,	7.5,	9,	12,	12,	0,	1.5,	15.0

Table 57d. Transferred adaptation to MCPA, followed by 2,4,5-T.

Key to columns in table: as in Table 57, above.

Perfusion started on 12/11/51 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried June 1951,) and 250 ml. of 100 ppm. MCPA solution, prepared from the "active" perfusate drained from a MCPA enriched perfuser (Table 48,) on 12/11/51. First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
0	67.0	75,	76,	81,	111,	13,	15,	19,	19,	3,	4.5,	75
3	75.2	60,	76,	83,	106,	15,	20,	23,	23,	3,	4,	65
5	75.2	75,	84,	87,	115,	15,	16,	16,	23,	3,	3,	90
7	81.6	47,	47,	65,	81,	14,	14,	14,	20,	2.5,	2.5,	100
9	69.5	50,	62,	65,	72,	25,	26,	27,	30,	3,	3,	90
11	59.4	51,	66,	88,	94,	25,	25,	40,	42,	3.5,	5,	55
13	55.9	108,	111,	124,	124,	34,	34,	36,	52,	7,	9,	30
15	68.3	53,	62,	70,	94,	26,	28,	29,	44,	4.5,	7.5,	40
17	66.0	81,	84,	92,	98,	39,	42,	47,	59,	6,	9,	25
19	67.4	107,	119,	119,	134,	36,	64,	65,	71,	9,	14,	10
22	67.6	92,	98,	99,	117,	50,	55,	55,	71,	16,	18,	8
24	70.7	74,	85,	88,	100,	74,	92,	95,	95,	65,	95,	<1
26	49.8	94,	98,	106,	136,	74,	94,	100,	104,	114,	164,	<1

Day 27. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

27a	70.3	56,	63,	66,	69,	17,	18,	20,	23,	3,	4.5,	70
30	72.4	68,	71,	72,	87,	21,	25,	25,	26,	3,	3,	50
32	67.8	78,	92,	92,	99,	24,	27,	43,	46,	4.5,	6,	35
34	60.3	104,	111,	113,	114,	55,	55,	58,	72,	8.5,	10,	20
38	74.3	66,	67,	72,	82,	47,	50,	50,	54,	108,	113,	<1
48	73.3	68,	75,	77,	102,	89,	92,	93,	104,	86,	116,	<1

Day 50. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

50a	74.3	54,	54,	61,	86,	13,	15,	16,	16,	2.5,	5.5,	90
53	70.8	61,	61,	82,	113,	20,	21,	30,	31,	3,	4,	65
55	65.5	102,	102,	106,	115,	23,	26,	32,	41,	4.5,	6,	40
57	72.0	97,	117,	117,	135,	31,	47,	57,	79,	7,	8.5,	20
59	71.6	91,	97,	98,	104,	77,	83,	102,	104,	69,	83,	<1
61b	60.8	86,	89,	104,	109,	95,	104,	112,	117,	118,	120,	<1

Day 61. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

61a	60.8	64,	68,	78,	82,	18,	21,	25,	31,	5,	6.5,	55
64	62.3	72,	77,	87,	95,	26,	30,	30,	30,	4.5,	4.5,	45
65	59.1	90,	95,	108,	132,	37,	41,	42,	44,	5,	7,	25
67	66.3	51,	53,	72,	92,	71,	86,	86,	107,	53,	80,	1
69b	51.2	117,	121,	123,	127,	72,	78,	88,	133,	123,	149,	<1

Table 57d. continued.

Day 69. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	G.
69a	51.2	64, 64, 76, 96,	18, 21, 23, 25,	4, 4,	65
72	43.0	79, 82, 86, 93,	33, 37, 37, 40,	14, 23,	25
75	58.4	101,115,123,156,	103,110,115,140,	64,124,	<1
77b	74.6	65, 68, 71, 84,	69, 71, 83, 92,	85, 89,	<1

Day 77. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

77a	86.6	45, 59, 59, 59,	13, 14, 15, 15,	2.5,2.5,	100
80	67.0	81, 97, 97,124,	33, 34, 45, 51,	4.5, 6,	35
82	46.6	105,129,142,151,	103,118,133,155,	75, 77,	<1
84b	65.0	59, 65, 77,112,	82, 83, 91,111,	88,114,	<1

Day 84. Perfuser drained and refilled with 250 ml. of 10 ppm. 2,4,5-T solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	H.
84a	65.0	94,102,102,112,	60, 65, 74, 93,	15, 19,	10.0
94	75.2	61, 76, 77,108,	84, 85, 85,107,	80, 97,	0.3
98	83.8	75, 87, 95,110,	79, 79, 87, 99,	75, 81,	0.5

Day 101. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4,5-T solution. Perfuser re-started, sampled after 1 hr.

A.	B.	D. D. D. D.	E. E. E. E.	F. F.	H.
101a	56.9	81, 84, 93,113,	16, 19, 21, 23,	3.5,5.5,	80
105	62.3	56, 74, 89,100,	17, 18, 21, 27,	3, 3,	90
110	53.4	64, 77, 77, 81,	15, 17, 19, 30,	4, 6,	70
115	71.1	80, 82, 92, 96,	15, 16, 23, 31,	3, 4,	70
120	69.9	80, 87, 89,121,	18, 21, 26, 33,	3, 3,	60
125	54.4	66, 68, 81, 85,	24, 24, 26, 26,	2,3.5,	60

Table 58. Transferred adaptation to MCPA, followed by 2,5-D.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Longest roots at nominal concentration of 10 ppm. as % m.c.
G. Indicated MCPA concentration in the perfusate (ppm.).
H. Indicated 2,5-D concentration in the perfusate (ppm.).

Perfusion started on 17/2/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 100 ppm. MCPA solution. This solution prepared from the combined "active" perfusates drained from two MCPA enriched perfusers,(Tables 49 and 59a) on 16 and 17/2/53 respectively. Solution common with that of a similar new perfuser which showed no sign of transferred adaptation in 35 days. Solution sampled before adding to the perfuser.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	G.
0	86.9	55, 56, 76, 78,	14, 14, 15, 15,	2.5,3.5,	100
10	94.5	60, 64, 76, 78,	14, 14, 15, 15,	1, 2,	100
20	81.6	78, 79, 85, 85,	29, 32, 34, 35,	3,4.5,	55
25b	84.1	63, 68, 75, 81,	77, 78, 85, 97,	81,119,	<1
Day 25. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.					
25a	78.7	57, 57, 60, 70,	12, 14, 15, 17,	2.5,2.5,	100
30	77.0	74, 79, 82, 97,	14, 16, 18, 23,	2.5, 4,	80
35	76.7	115,117,120,128,	98, 98,106,108,	108,109,	<1
38b	77.8	109,112,113,116,	102,103,107,113,	93,102,	<1
Day 38. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.					
38a	77.8	63, 65, 68, 68,	19, 21, 22, 23,	1.5,2.5,	90
41	77.1	69, 70, 72, 72,	18, 19, 21, 28,	4, 5,	75
44	85.2	65, 68, 70, 70,	23, 23, 23, 33,	4.5, 6,	60
47	74.7	83, 90, 94, 95,	100,104,107,112,	68, 82,	1
Day 50. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.					
50a	83.4	38, 47, 47, 63,	14, 15, 16, 17,	2.5,2.5,	100
53	76.0	55, 56, 63, 73,	17, 19, 21, 28,	2.5,5.5,	80
56	80.0	91, 93, 99,110,	83, 88, 88, 89,	71, 85,	1
59	81.7	70, 74, 76, 83,	71, 77, 78, 82,	79, 89,	<1

Table 58. continued.

Day 59. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	G.
59a	81.7	45, 46, 48, 66,	13, 14, 16, 16,	1,2.5,	100
62	73.9	84, 95,107,111,	46, 51, 53, 58,	12, 14,	25
65	76.7	90, 94, 96, 99,	106,106,108,111,	86, 94,	<1

Day 68. Perfuser drained and refilled with 250 ml. of 10 ppm. 2,5-D solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	H.
68a	69.9	119,123,126,127,	88, 96, 97,106,	30, 40,	8.0
74	75.5	86, 91, 94, 96,	80, 82, 94,108,	28, 32,	10.0
80	80.6	115,115,123,123,	82, 84, 88, 89,	36, 47,	6.5
86	75.1	82, 89, 93,106,	85, 86, 92, 94,	40, 45,	5.0
92	90.0	88, 92, 98,101,	61, 62, 73, 77,	36, 39,	7.0
98	92.6	87, 87, 89, 94,	85, 87, 88, 88,	38, 38,	6.0
104	93.9	89, 91, 93, 95,	93, 93, 97,104,	35, 46,	4.0
110	98.6	91, 95, 96,102,	96, 97,101,103,	42, 52,	3.0
116	88.3	93, 98,100,105,	93, 94, 95,102,	43, 70,	2.5
122	94.4	87, 91, 97, 98,	92, 93, 97, 99,	59, 62,	2.0
128	100.1	92,102,102,103,	84, 84, 85,101,	70, 74,	1.5
134	101.4	92, 95, 96,102,	94, 97,101,105,	60, 66,	1.5

Table 58a. Transferred adaptation to MCPA, followed by 2,5-D.

Key to columns in table: as for Table 58, above.

Perfusion started on 19/4/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 100 ppm. MCPA solution prepared from the combined "active" perfusates drained from two MCPA enriched perfusers (Tables 59 and 60b) on 19/4/53. Solution common with that of Table 52.
First sample taken after 1 hr. perfusion.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	G.
0	74.9	72, 73, 77, 80,	17, 18, 25, 28,	1.5,1.5,	95
6	74.9	58, 59, 69, 80,	14, 15, 17, 17,	1.5, 3,	95
12	81.4	59, 59, 62, 78,	11, 11, 14, 14,	1,2.5,	110
18	80.6	54, 61, 70, 73,	16, 16, 18, 19,	1, 1,	90
24	80.7	88, 94, 98, 99,	25, 26, 31, 31,	3.5,3.5,	40
30	87.6	95,103,105,106,	90, 95,101,105,	80, 85,	<1

Table 58a. continued.

Day 33. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	G.
33a	88.3	59, 62, 63, 70,	16, 18, 18, 18,	2.5,2.5,	90
36	89.8	69, 73, 83, 83,	13, 14, 15, 16,	2,3.5,	100
39	95.0	93, 96, 96, 98,	76, 78, 78, 82,	26, 26,	10
42b	96.9	93, 93, 99,101,	94, 95, 96, 98,	99,102,	<1

Day 42. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

42a	96.9	71, 76, 76, 82,	13, 14, 15, 16,	1, 1,	100
45	100.4	91, 91, 93,102,	60, 61, 62, 63,	8, 10,	15
48b	95.7	98, 98,100,101,	98, 98, 99, 99,	100,101,	<1

Day 48. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

48a	95.7	66, 66, 73, 78,	15, 16, 17, 18,	1, 1,	85
51	100.5	95,106,107,107,	91, 91, 97,104,	100,102,	<1

Day 54. Perfuser drained and refilled with 250 ml. of 10 ppm. 2,5-D solution. Perfuser re-started, sampled after 1 hr.

A.	B.	D. D. D. D.	E. E. E. E.	F. F.	H.
54a	91.9	68, 69, 72, 83,	26, 27, 27, 30,	7.5, 12,	10.0
74	97.5	99,100,103,105,	50, 54, 62, 72,	14, 18,	2.5
84	95.3	94, 94,103,104,	69, 76, 76, 76,	24, 25,	1.25
94	102.6	102,104,109,111,	91, 93,101,103,	35, 40,	0.5
114	102.3	97, 98,109,110,	94, 95, 95, 96,	93,100,	<0.01

Table 59. Transferred adaptation to MCPA, followed by 2,4-DM.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Longest roots at nominal concentration of 10 ppm. as % m.c.
G. Indicated MCPA concentration in the perfusate (ppm.).
H. Indicated 2,4-DM concentration in the perfusate (ppm.).

Perfusion started on 8/2/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 100 ppm. MCPA solution prepared from the combined "active" perfusates drained from two MCPA enriched perfusers (Tables 59a and 60,) on 8/2/53. Solution common with that in Table 60b. First sample taken before adding solution to the perfuser.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
0	84.2	43,	45,	50,	51,	12,	12,	12,	13,	2.5,	2.5,	120
10	82.6	53,	58,	62,	75,	11,	13,	14,	16,	2.5,	3.5,	120
20	86.1	70,	72,	75,	90,	33,	35,	35,	41,	6,	7,	40
25b	83.5	72,	74,	86,	87,	27,	37,	38,	50,	7,	8.5,	35

Day 25. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

25a	83.5	44,	49,	55,	63,	13,	15,	18,	19,	1,	2.5,	90
28	76.0	78,	78,	83,	85,	14,	15,	20,	20,	1.5,	2.5,	80
31	92.9	51,	52,	52,	71,	14,	15,	15,	16,	1,	2,	100
34	84.1	49,	51,	59,	60,	15,	18,	20,	20,	3.5,	6,	70
37	82.7	75,	91,	94,	95,	34,	38,	39,	40,	7,	8.5,	30
40	71.1	91,	96,	108,	113,	94,	97,	98,	124,	63,	70,	1.5

Day 43. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

43a	73.9	53,	58,	64,	69,	15,	15,	17,	18,	2.5,	4,	100
46	79.9	53,	59,	74,	86,	14,	14,	15,	16,	2.4,	2.5,	100
49	88.3	80,	82,	85,	86,	28,	29,	31,	32,	8,	9,	30
52	82.5	75,	83,	95,	105,	78,	83,	85,	86,	65,	71,	1.5
55b	79.3	60,	62,	64,	69,	101,	101,	104,	104,	81,	100,	<1

Day 55. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

55a	79.3	54,	55,	57,	57,	14,	14,	17,	18,	1.5,	1.5,	100
58	86.7	43,	44,	49,	49,	15,	17,	17,	20,	2.5,	3.5,	90
61	82.3	75,	79,	82,	91,	82,	82,	85,	109,	58,	62,	1.5
64b	77.9	76,	77,	77,	94,	83,	92,	95,	104,	91,	110,	<1

Table 59. continued.

Day 64. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
64a	77.9	48,	51,	54,	58,	15,	16,	17,	18,	2.5,	2.5,	100
70b	74.9	93,	99,	108,	111,	89,	91,	91,	109,	67,	68,	1

Day 70. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-DM solution. Perfuser re-started, sampled after 1 hr.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	H.
70a	74.9	61,	63,	64,	69,	18,	21,	23,	24,	1.5,	1.5,	80
80	81.9	55,	65,	66,	71,	19,	22,	24,	26,	2.5,	2.5,	70
90	84.3	58,	60,	60,	70,	15,	16,	17,	18,	1,	1,	100
100	87.6	48,	49,	51,	56,	18,	19,	20,	23,	2.5,	4.5,	95
110	93.3	66,	74,	76,	77,	17,	18,	19,	19,	1,	2,	90
120b	95.2	63,	63,	66,	75,	25,	25,	27,	29,	6.5,	8.5,	65

Day 120. Perfuser drained and refilled with 250 ml. of 10 ppm. 2,4-DM solution. Perfuser re-started, sampled after 1 hr.

120a	95.2	45,	45,	46,	52,	13,	16,	23,	25,	2,	2,	9.5
140	102.9	62,	71,	72,	83,	16,	17,	18,	20,	2,	3,	10.0
148	98.6	69,	71,	73,	74,	21,	27,	33,	36,	2,	3,	5.5

Table 59a. Transferred adaptation to MCPA, followed by 2,4-DM.

Key to columns in Table: as in Table 59, above.

Perfusion started on 18/11/52 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried February 1952,) and 250 ml. of 100 ppm. MCPA solution, prepared from the "active" perfusate drained from an MCPA enriched perfuser(Table 60a,) on 18/11/52. Solution common with that in Table 60. First sample taken after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
0	81.2	43,	46,	46,	54,	19,	20,	22,	22,	2.5,	3.5,	65
10	63.7	70,	80,	83,	94,	22,	27,	30,	38,	3,	4.5,	60
15	45.3	49,	51,	55,	55,	20,	22,	24,	29,	2,	4.5,	65
20	81.6	62,	68,	68,	79,	20,	24,	26,	28,	4.5,	6,	55
25	75.4	76,	77,	86,	88,	20,	27,	29,	32,	4,	4,	55
29	77.1	69,	74,	75,	100,	32,	32,	39,	44,	9,	11,	25
55	68.0	94,	105,	106,	110,	87,	88,	96,	103,	81,	103,	<1
58	78.1	70,	78,	79,	90,	79,	86,	92,	100,	83,	90,	<1

Table 59a. continued.

Day 58. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	G.
58a	78.1	40, 40, 44, 47,	15, 16, 17, 19,	1.5,2.5,	95
61	67.8	38, 41, 46, 47,	14, 16, 18, 24,	1.5, 3,	95
64	76.1	63, 72, 76, 77,	19, 22, 22, 24,	2.5, 4,	65
67	79.2	67, 72, 78, 83,	25, 35, 35, 45,	5,6.5,	35
70	80.5	103,104,109,114,	87, 88, 93, 96,	100,105,	<1
73b	76.6	85, 91, 92, 95,	81, 86, 90, 92,	76, 94,	<1

Day 73. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA. Perfuser re-started, sampled after 1 hr.

73a	76.6	36, 44, 48, 53,	13, 13, 16, 17,	2.5,2.5,	105
76	87.8	50, 51, 59, 64,	16, 17, 18, 22,	2.5,4.5,	90
79	85.9	92, 94,101,102,	86, 87, 97,105,	99,107,	<1
82b	84.2	92,102,104,111,	98, 98,103,111,	78, 95,	<1

Day 82. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

82a	84.2	42, 45, 52, 53,	13, 14, 15, 18,	2.5,2.5,	105
85	86.5	60, 67, 70, 71,	19, 22, 24, 30,	3.5, 7,	65
88	78.0	90, 91, 99, 99,	73, 73, 77,102,	76, 93,	1
91b	86.9	90, 93,107,108,	101,101,104,104,	105,111,	<1

Day 91. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-DM solution. Perfuser re-started, sampled after 1 hr.

A.	B.	D. D. D. D.	E. E. E. E.	F. F.	H.
91a	86.9	50, 51, 53, 54,	12, 13, 15, 21,	1,2.5,	130
97	84.1	39, 41, 46, 51,	12, 12, 12, 14,	1, 2,	170
103	82.0	46, 49, 52, 58,	22, 22, 23, 23,	2.5,2.5,	100
109	67.8	52, 56, 65, 68,	16, 17, 19, 21,	3,4.5,	100
115	81.9	48, 57, 58, 61,	17, 18, 21, 21,	10, 11,	100
121b	77.0	34, 36, 52, 64,	13, 14, 16, 18,	2.5, 4,	130

Day 121. Perfuser drained and refilled with 250 ml.of 10 ppm. 2,4-DM solution. Perfuser re-started, sampled after 1 hr.

121a	71.1	32, 35, 38, 44,	14, 18, 20, 27,	1.5, 3,	9.5
131	88.3	57, 59, 64, 68,	13, 15, 17, 19,	1,3.5,	10.5
141	81.9	46, 46, 51, 54,	12, 13, 14, 15,	1, 1,	13.0
151	74.6	44, 45, 45, 47,	12, 14, 16, 19,	2.5,2.5,	13.0
161	78.5	74, 79, 84, 88,	32, 33, 34, 48,	2.5,2.5,	4.0
171	80.6	88, 89, 98,118,	48, 58, 70, 74,	17, 19,	1.0
181	80.8	105,112,115,119,	98,101,101,120,	94,102,	<0.1

Table 60. Transferred adaptation to MCPA, followed by 2,4-DM.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Longest roots at nominal concentration of 10 ppm. as % m.c.
G. Indicated MCPA concentration in the perfusate (ppm.).
H. Indicated 2,4-DM concentration in the perfusate (ppm.).

Perfusion started on 18/11/52 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried February 1952,) and 250 ml. of 100 ppm. MCPA solution prepared from the"active" perfusate drained from an MCPA enriched perfuser (Table 60a,) on 18/11/52. Solution common with that in Table 59a. First sample taken after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
0	81.2	48,	50,	63,	69,	15,	16,	17,	19,	1,	2.5,	90
10	63.7	74,	75,	80,	100,	22,	25,	25,	25,	1.5,	1.5,	55
15	45.3	53,	55,	57,	69,	17,	22,	27,	27,	4.5,	4.5,	65
20	81.6	56,	71,	71,	73,	18,	19,	22,	28,	2.5,	3.5,	70
25	75.4	84,	85,	95,	96,	23,	29,	35,	41,	4,	8,	40
29	77.1	82,	86,	86,	95,	60,	61,	62,	63,	19,	22,	7
55	68.0	84,	85,	88,	107,	88,	90,	91,	98,	94,	96,	<1
58b	78.1	74,	76,	87,	88,	84,	84,	92,	93,	76,	82,	<1

Day 58. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

58a	78.1	36,	37,	44,	45,	13,	13,	15,	16,	1.5,	2.5,	105
61	67.8	43,	46,	47,	55,	16,	16,	17,	19,	1.5,	3,	90
64	76.1	45,	47,	55,	56,	17,	18,	22,	25,	1.5,	2.5,	70
67	79.2	38,	42,	42,	45,	23,	32,	34,	40,	4,	6.5,	40
70	80.5	89,	89,	93,	108,	95,	103,	112,	117,	84,	88,	<1
73b	76.6	77,	87,	90,	109,	78,	82,	87,	95,	72,	85,	<1

Day 73. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

73a	76.6	43,	46,	49,	52,	14,	15,	17,	22,	1.5,	2.5,	100
76	87.8	60,	66,	67,	72,	24,	30,	31,	31,	3.5,	4.5,	50
79	85.9	76,	77,	79,	88,	35,	35,	36,	55,	9.5,	11,	30
82b	84.2	89,	90,	94,	101,	97,	100,	105,	115,	94,	100,	<1

Day 82. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

82a	84.2	40,	44,	51,	65,	13,	14,	15,	16,	2.5,	2.5,	105
85	86.5	45,	56,	65,	82,	27,	27,	28,	31,	3.5,	3.5,	50
88	78.0	74,	79,	82,	95,	74,	77,	82,	82,	74,	91,	<1

Table 60. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
91b	86.9	86,	87,	91,	95,	91,	93,	95,	95,	84,	90,	1

Day 91. Perfuser drained and refilled with 250 ml. of 10 ppm. 2,4-DM solution. Perfuser re-started, sampled after 1 hr.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	H.
91a	86.9	43,	48,	51,	57,	14,	15,	16,	20,	1,	2.5,	12.0
94	79.3	66,	67,	69,	69,	15,	16,	19,	25,	2.5,	4,	9.0
97	84.1	52,	52,	59,	76,	15,	16,	16,	17,	3.5,	4.5,	12.0
100	88.2	85,	88,	92,	103,	31,	32,	34,	36,	9,	10,	3.5
103	82.0	66,	68,	83,	89,	51,	54,	56,	61,	14,	16,	1.25
106	86.1	82,	90,	93,	95,	67,	70,	72,	74,	14,	20,	1.0
109	67.8	90,	96,	97,	97,	72,	85,	88,	91,	16,	21,	0.5
112	91.3	79,	81,	85,	93,	73,	87,	90,	103,	41,	50,	0.2
115	81.9	76,	85,	90,	93,	68,	74,	80,	106,	65,	73,	<0.1
118	71.0	73,	84,	96,	101,	100,	103,	106,	107,	77,	86,	<0.1

Day 121. Perfuser drained and refilled with 250 ml. of 10 ppm. 2,4-DM solution. Perfuser re-started, sampled after 1 hr.

121a	71.1	53,	53,	53,	64,	17,	17,	18,	19,	3,	3,	10.0
131	88.3	82,	88,	88,	94,	44,	49,	52,	60,	13,	16,	1.5
141	81.9	88,	91,	93,	98,	77,	78,	100,	103,	46,	52,	0.2
146b	77.9	58,	74,	81,	82,	69,	74,	80,	89,	80,	81,	<0.1

Day 146. Perfuser drained and refilled with 250 ml. of 10 ppm. 2,4-DM solution. Perfuser re-started, sampled after 1 hr.

146a	77.9	37,	37,	40,	45,	14,	15,	16,	21,	1.5,	2.5,	12.0
151	74.6	95,	98,	100,	102,	29,	32,	35,	47,	4,	11,	3.0
161	78.5	105,	108,	109,	113,	104,	105,	105,	119,	104,	117,	<0.1
166b	75.5	99,	102,	106,	107,	92,	96,	102,	102,	94,	108,	<0.1

Day 166. Perfuser drained and refilled with 250 ml. of 10 ppm. 2,4-DM solution. Perfuser re-started, sampled after 1 hr.

166a	75.5	40,	40,	45,	57,	14,	16,	19,	21,	2.5,	2.5,	10.0
170	83.9	86,	87,	88,	92,	29,	31,	31,	32,	2.5,	7,	5.0
174	88.6	90,	90,	91,	97,	38,	38,	39,	42,	3.5,	9,	3.0
178	75.1	112,	113,	118,	128,	40,	43,	44,	57,	18,	19,	1.5
182	87.6	89,	90,	102,	103,	80,	81,	81,	84,	29,	32,	0.4
186	88.3	92,	96,	97,	100,	97,	98,	100,	103,	45,	56,	0.2
190	95.0	91,	96,	98,	105,	88,	89,	96,	110,	69,	78,	<0.1
194b	93.9	89,	95,	97,	106,	98,	101,	109,	111,	104,	105,	<0.1

Day 194. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-DM solution. Perfuser re-started, sampled after 1 hr.

194a	96.9	56,	56,	59,	65,	18,	19,	25,	25,	4,	5,	90
204	95.2	66,	66,	67,	89,	24,	25,	29,	30,	4,	6.5,	60
214	103.4	70,	74,	77,	82,	31,	36,	37,	39,	4,	7.5,	45
224	102.7	67,	71,	71,	74,	25,	27,	30,	32,	8,	8.5,	40

Table 60a. Transferred adaptation to MCPA, followed by 2,4-DM.

Key to columns in table: as in Table 60, above.

Perfusion started on 25/9/52 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried February 1952,) and 250 ml. of 100 ppm. MCPA solution prepared from the combined "active" perfusates drained from two MCPA enriched perfusers (Tables 51a and 57b,) on 25/9/52. First sample taken after 15 days perfusion.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	G.
15	67.4	47, 50, 62, 64,	15, 15, 17, 20,	1.5,1.5,	95
18	64.8	39, 42, 57, 82,	12, 14, 19, 20,	3, 3,	90
21	76.5	65, 68, 75, 82,	21, 22, 24, 27,	2.5, 4,	80
24	76.3	83, 83, 96, 96,	26, 28, 33, 33,	4, 4,	45
27	80.3	73, 74, 74, 88,	45, 46, 48, 52,	11, 14,	20
30	76.6	89, 94,101,120,	91, 94, 95,101,	88, 90,	<1
32b	72.5	84, 87, 91,106,	84,101,106,106,	81, 88,	<1

Day 32. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

32a	65.4	50, 52, 60, 72,	18, 19, 20, 20,	3, 3,	70
36	68.6	55, 57, 58, 61,	17, 19, 19, 22,	3,4.5,	70
39	65.2	74, 74, 78, 78,	31, 32, 35, 41,	4.5, 6,	45
42	50.0	100,108,116,144,	106,108,110,112,	90,112,	<1
45b	65.0	89, 91, 97,101,	83, 91, 91, 95,	46, 74,	1

Day 45. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

45a	60.6	66, 68, 71, 78,	20, 20, 21, 21,	1.5,1.5,	65
48	64.7	73, 76, 84,119,	37, 37, 39, 43,	9.5, 11,	20
51	74.4	82, 87, 93,105,	92, 92, 98, 98,	74, 82,	1
54b	75.1	85, 94, 97,104,	85, 89, 91, 93,	95, 95,	1

Day 54. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

54a	81.2	55, 59, 64, 73,	17, 18, 20, 22,	1, 1,	70
57	66.5	90, 93, 95,113,	27, 29, 30, 42,	1.5,4.5,	40
60b	79.2	93, 95, 97,109,	101,106,107,115,	49, 79,	1

Day 60. Perfuser drained and refilled with 250 ml. of 10 ppm. 2,4-DM solution. Perfuser re-started, sampled after 1 hr.

A.	B.	D. D. D. D.	E. E. E. E.	F. F.	H.
60a	79.2	18, 21, 23, 38,	4, 5, 5,6.5,	1.5,2.5,	8.0
63	77.9	76, 76, 81, 95,	42, 46, 53, 55,	17, 19,	1.5
66b	64.0	95,102,105,105,	97,100,105,108,	91, 98,	<1

Table 60a. continued.

Day 66. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-DM solution. Perfuser re- started, sampled after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	H.
66a	64.0	84,	88,	91,	103,	63,	64,	66,	66,	9.5,	13,	90
69	45.3	86,	86,	95,	102,	75,	75,	86,	95,	27,	29,	55
72	62.9	81,	83,	89,	100,	45,	46,	49,	52,	16,	18,	110
75	79.7	102,	103,	104,	108,	55,	56,	56,	60,	15,	19,	120
78	85.1	88,	88,	92,	93,	58,	61,	67,	68,	17,	21,	90
81	65.0	89,	92,	94,	100,	63,	63,	71,	74,	14,	14,	95
83	66.6	75,	78,	78,	81,	66,	71,	75,	80,	21,	27,	70
104	66.6	90,	95,	101,	102,	57,	68,	77,	78,	19,	21,	90
107	65.3	79,	79,	79,	96,	52,	57,	57,	57,	21,	23,	110
110	68.5	70,	73,	74,	92,	63,	70,	70,	80,	16,	20,	85
115	67.5	75,	78,	80,	93,	53,	62,	65,	77,	21,	22,	85
120	79.2	86,	87,	92,	96,	59,	60,	64,	71,	16,	20,	100
125	76.6	59,	68,	68,	74,	72,	72,	72,	73,	17,	21,	90
130	87.1	88,	91,	93,	100,	65,	65,	65,	65,	12,	16,	80

Table 60b. Transferred adaptation to MCPA, followed by 2,4-DM.

Key to columns in table: as in Table 60, above.

Perfusion started on 8/2/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 100 ppm. MCPA solution prepared from the combined "active" perfusates drained from two MCPA enriched perfusers (Tables 59a and 60,) on 8/2/53. Solution common with that in Table 59. First sample taken before adding solution to the perfuser.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
0	84.2	43,	45,	50,	51,	12,	12,	12,	13,	2.5,	2.5,	120
10	82.6	48,	50,	62,	63,	11,	14,	17,	18,	2.5,	2.5,	105
20	86.1	50,	59,	67,	84,	24,	24,	26,	34,	6,	8,	65
25b	83.5	69,	76,	82,	90,	35,	41,	48,	51,	8.5,	11,	30

Day 25. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

25a	83.5	56,	59,	60,	75,	12,	12,	13,	14,	1,	2.5,	120
28	76.0	63,	64,	64,	66,	13,	14,	15,	16,	1.5,	2.5,	110
31	92.9	61,	62,	71,	72,	14,	15,	18,	20,	2,	4.5,	90
34	84.1	48,	51,	63,	77,	16,	18,	18,	20,	5,	6,	80
37	82.7	89,	91,	99,	100,	89,	91,	94,	103,	31,	40,	40
40	71.1	67,	73,	77,	107,	90,	90,	101,	113,	73,	110	<1

Table 60b. continued.

Day 43. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	G.
43	73.9	51, 55, 65, 65,	15, 17, 18, 24,	1.5,2.5,	100
46	79.9	88, 90, 90,105,	16, 17, 18, 19,	2.5,2.5,	85
49	88.3	95,100,104,106,	28, 28, 29, 31,	7, 8,	50
52	82.5	82, 85, 88, 94,	89, 91, 95, 97,	94, 95,	<1
55b	79.3	79, 93, 98,106,	83, 92, 93, 97,	77, 93,	<1

Day 55. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

55a	79.3	38, 45, 48, 50,	11, 12, 13, 14,	1.5,2.5,	120
58	86.7	63, 65, 73, 78,	21, 22, 23, 24,	3.5,4.5,	65
61	82.3	75, 78, 81, 86,	91, 92, 93, 96,	67, 72,	1
64b	77.9	68, 69, 74, 96,	92,100,101,112,	81,122,	<1

Day 64. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

64a	77.9	50, 52, 59, 64,	14, 15, 15, 16,	1.5,2.5,	100
70b	74.9	105,105,107,120,	99,103,104,112,	89,107,	<1

Day 70. Perfuser drained and refilled with 250 ml. of 10 ppm. 2,4-DM solution. Perfuser re-started, sampled after 1 hr.

A.	B.	D. D. D. D.	E. E. E. E.	F. F.	H.
70a	74.9	56, 57, 59, 64,	13, 16, 16, 18,	0,2.5,	12.0
80	81.9	92, 93,110,114,	23, 24, 24, 29,	14, 16,	1.2
90	84.3	86, 92, 96, 96,	56, 62, 62, 70,	18, 20,	1.0
100b	87.6	89, 90,103,104,	48, 51, 56, 63,	12, 13,	1.5

Day 100. Perfuser drained and refilled with 250 ml. of 10 ppm. 2,4-DM solution. Perfuser re-started, sampled after 1 hr.

100a	87.6	59, 59, 60, 63,	15, 15, 18, 19,	1,2.5,	11.0
104	88.3	74, 79, 80, 83,	18, 18, 26, 26,	5.5, 7,	4.0
108	95.0	57, 58, 59, 70,	12, 13, 14, 19,	3, 3,	11.0
112	96.9	79, 80, 87, 87,	21, 22, 25, 28,	3, 4,	6.5
120	95.2	84, 84, 85, 86,	21, 23, 27, 27,	3, 4,	6.5

Table 61. Transferred adaptation to MCPA, followed by 2,4-DCP.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Indicated MCPA concentration in the perfusate (ppm.).
G. Colourimeter reading (E.E.L. instrument,) in divisions.
H. Indicated 2,4-DCP concentration in the perfusate (ppm.).

Perfusion started on 21/2/52 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1952,) and 250 ml. of 10.0 ppm. MCPA solution prepared from the "active" perfusate drained from a MCPA enriched perfuser (Table 57c,) on 20/2/52. First sample taken after 1 hr. perfusion.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
0	56.9	60, 78, 83,106,	67, 71, 78, 88,	14, 16,	10.0
3	63.5	85, 87, 98,110,	50, 54, 71, 95,	11, 13,	12.0
6	67.4	64, 76, 98, 99,	70, 73, 92, 94,	11, 11,	9.0
8	55.1	100.104,122,122,	85, 87, 96, 98,	9, 35,	5.0
11	61.2	105,113,121,124,	61, 62, 66, 69,	19, 25,	1.3
13	58.0	105,105,121,135,	81, 86, 92,112,	22, 41,	0.5
15	71.1	82, 85, 86, 96,	86, 90,104,115,	37, 41,	0.3
17	67.5	104,107,108,144,	52, 62, 90, 92,	84, 86,	<0.1
19	71.9	91, 91,103,116,	109,112,116,117,	53, 82,	0.1

Day 21. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

21a	70.0	81, 89, 93, 96,	19, 19, 24, 26,	1.5, 3,	80
23	65.3	66, 68, 68, 78,	20, 22, 22, 25,	1.5, 3,	80
25	64.4	56, 62, 79, 82,	17, 20, 20, 33,	3,4.5,	70
27	72.5	73, 84, 89, 94,	21, 22, 28, 39,	3, 4,	70
29	62.8	53, 56, 58, 79,	19, 22, 27, 30,	3, 5,	60
31	69.6	77, 86, 86, 89,	34, 34, 43, 47,	3.5,4.5,	55
33	60.3	92, 92, 93,107,	35, 38, 58, 72,	6.5, 10,	35
35	56.0	77, 84,106,125,	23, 25, 41, 43,	9, 13,	30
38	69.6	62, 89,118,122,	40, 42, 49, 63,	7, 13,	25
40	60.9	76, 79, 89,120,	66, 69, 73, 84,	18, 20,	10
42	66.6	84, 84, 92,111,	80, 93,102,111,	42, 48,	3
44	59.8	62, 64, 77,121,	87, 97,100,102,	42, 70,	2

Day 46. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

46a	79.0	66, 71, 72, 80,	14, 14, 14, 17,	4,6.5,	110
48	79.0	61, 72, 76, 96,	13, 14, 14, 18,	2.5,2.5,	110
66	69.8	63, 74,100,132,	30, 33, 39, 40,	4.5, 7,	40
69	72.4	67, 97,107,120,	43, 43, 50, 54,	4,8.5,	30

Table 61. continued.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
72	79.1	86, 92,106,119,	83, 91, 92,110,	40, 44,	3
75	60.8	79, 92, 92,112,	61, 63, 79,117,	49,109,	<1

Day 78. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

78a	58.3	60, 60, 62, 71,	21, 21, 22, 22,	3.5,3.5,	70
81	56.6	76, 78, 83,115,	23, 25, 30, 32,	5.5, 9,	50
84	60.0	53, 63, 67, 68,	40, 43, 43, 50,	13, 15,	30
87	54.4	64, 83, 85, 90,	76, 96,103,105,	18, 29,	10
90	71.0	69, 69, 73,113,	52, 73, 78, 89,	49, 55,	2
93	64.0	81,103,103,108,	100,105,105,130,	28, 61,	1
96	61.6	81, 81, 94,104,	98,117,120,125,	85,108,	1

Day 99. Perfuser drained and refilled woth 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

99a	47.3	34, 47, 68, 76,	30, 32, 32, 45,	6.5,6.5,	40
102	54.5	59, 62, 70, 88,	24, 28, 28, 28,	5.5,7.5,	50
105	49.4	59, 63, 83, 93,	28, 28, 28, 37,	6, 12,	40
108	48.9	98,106,119,123,	72, 82, 84, 90,	123,139,	<1

Day 109. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Perfuser re-started. Solution sampled before adding to perfuser and after 1 hr. perfusion.

A.	G.	H.	A.	G.	H.	A.	G.	H.
109ba	94.5	189,	114	43.0	86,	120	27	54,
109alhr	72.5	145,	115	41.5	83,	121	20	40,
110	66.0	132,	116	39.1	78,	122	/	/,
111	55.5	111,	117	34.1	68,	123	10	20,
112	54.0	108,	118	33.0	66,	124b	10	20,
113	47.5	95,	119	32.0	64,			

Day 124. Perfuser drained and refilled with 250 ml. of 100 ppm 2,4-DCP solution. Sampled before adding to perfuser and after 1hr

124ba	48.3	97,	125	23.8	48,	127	14.5	29,
124alhr	43.4	87,	126	20.7	41,	128b	9.4	19,

Day 124. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-DCP solution. Sampled before adding to perfuser and after 1hr

128ba	57.0	114,	129	32.7	66,	130b	14.1	28,
128alhr	43.0	86,						

Day 130. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-DCP solution. Sampled before adding to perfuser and after 1hr

130ba	58.0	116,	131	37.5	75,	133	6.6	13,
130alhr	50.4	101,	132	25.0	50,			

Table 61a. Transferred adaptation to MCPA, followed by 2,4-DCP.

Key to columns in table: as in Table 61.

Perfusion started on 20/3/52 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1952,), and 250 ml. of 100 ppm. MCPA solution prepared from the "active" perfusate drained from a MCPA enriched perfuser (Table 57c,) on 18/3/52. First sample taken after 1 hr. perfusion.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	F.
0	63.1	60, 65, 86,111,	14, 16, 16, 24,	1.5, 3,	100
5	60.3	52, 68, 78, 87,	18, 20, 22, 25,	1.5, 3,	70
8	74.2	44, 57, 73, 88,	13, 14, 15, 16,	1.5,2.5,	100
11	68.3	58, 67, 68, 80,	13, 15, 15, 16,	1.5, 3,	100
14	66.6	69, 71, 81,107,	15, 15, 16, 18,	1.5, 3,	95
17	58.8	89, 94, 99,128,	15, 17, 17, 22,	3.5, 7,	80
20	79.0	88, 94,100,106,	32, 33, 34, 37,	4,6.5,	40
38	69.8	109,109,124,125,	69, 84,122,133,	56, 57,	2
40	69.3	96,103,116,121,	79, 93, 95, 99,	92,106,	1

Day 41. Perfuser drained and refilled with 250 ml. of 10 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

41a	69.8	74, 84, 86,112,	32, 34, 36, 37,	4.5, 6,	4.0
44	79.1	85, 85, 87, 89,	21, 25, 30, 35,	2.5, 4,	6.5
47	60.8	79, 95,109,125,	63, 84, 95,119,	89,110,	<1

Day 50. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

50a	58.3	57, 58, 72, 74,	17, 19, 19, 26,	1.5,3.5,	85
53	56.6	51, 62, 71, 72,	21, 23, 27, 27,	3.5,5.5,	65
56	60.0	65, 73, 78, 82,	20, 20, 30, 37,	3.5, 5,	55
59	54.4	83, 83,110,129,	85, 98,105,129,	64, 74,	1.0
62	71.0	86, 86,101,123,	58, 69,103,107,	52, 82,	1.0
65	64.0	66, 69, 71, 99,	58, 58, 64, 81,	67,103,	<1

Day 68. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

68a	61.6	55, 57, 60, 62,	11, 14, 18, 21,	3.5,3.5,	100
71	47.3	79, 81, 98,121,	57, 59, 64,114,	30, 32,	45
74	54.5	90, 92, 97,103,	53, 64, 68, 70,	9, 11,	10
77	49.4	73, 79,101,132,	63, 63, 65, 67,	36, 39,	3.5
80	48.9	78, 88, 92,108,	88, 90, 96,125,	31, 61,	3

Day 81. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding to the perfuser and after 1 hr. perfusion.

A.	G.	H.	A.	G.	H.	A.	G.	H.
81ba	94.5	189,	82	64.5	129,	84	52	104,
81alhr	76.5	153,	83	51.5	103,	85	48	96,

Table 61a. continued.

A.	G.	H.	A.	G.	H.	A.	G.	H.
86	45	90,	90	36.2	72,	94	/	/,
87	41.2	82,	91	33	66,	95	14	28,
88	40.3	81,	92	29.5	59,	96b	13	26,
89	35.4	71,	93	24.2	48,			

Day 96. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-DCP solution. Solution sampled before adding to the perfuser and after 1 hr. perfusion.

96ba	48.3	97,	99	26.3	53,	103	10.7	21,
96alhr	39.3	79,	100	23.3	46.5,	104	9.4	19,
97	31.6	63,	101	17.3	35.5,	105	5.3	11,
98	30.6	61,	102	13.6	27,			

Table 61b. Transferred adaptation to MCPA, followed by 2,4-DCP.

Key to columns in table: as in Table 61.

Perfusion started on 11/8/51 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried June 1951,) and 250 ml. of 100 ppm. MCPA solution prepared from the "active" perfusate drained from a MCPA enriched perfuser (Table 48,) on 11/8/51. First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	62.2	71,	71,	89,	100,	21,	29,	31,	48,	3,	3,	65
3	49.7	79,	81,	109,	123,	34,	38,	38,	40,	4,	6,	40
6	67.4	74,	76,	85,	97,	19,	19,	19,	36,	3,	6,	65
9	64.1	75,	80,	84,	98,	17,	22,	25,	28,	3,	3,	70
11	58.7	75,	79,	82,	84,	22,	26,	27,	29,	3.5,	3.5,	65
13	70.0	80,	90,	103,	103,	19,	20,	27,	31,	3,	6,	50
15	67.2	75,	82,	82,	88,	18,	18,	22,	24,	3,	4.5,	65
17	72.8	74,	85,	103,	117,	26,	29,	33,	34,	5.5,	5.5,	40
19	62.6	83,	90,	95,	99,	24,	26,	35,	46,	3,	5,	35
21	58.6	63,	67,	68,	73,	20,	20,	20,	23,	3,	5,	60
23	60.1	108,	115,	120,	130,	28,	30,	32,	33,	5,	5,	40
25	51.6	103,	103,	108,	110,	31,	33,	33,	37,	6,	18,	30
27	51.8	81,	87,	97,	114,	29,	31,	31,	39,	6,	6,	35
29	42.3	102,	102,	118,	132,	52,	57,	66,	80,	7,	9.5,	15
31	45.4	86,	115,	135,	137,	73,	79,	86,	93,	15,	20,	7
33	46.2	70,	83,	87,	98,	84,	87,	87,	89,	67,	74,	1
35	49.2	118,	134,	137,	175,	118,	118,	124,	134,	98,	110,	<1

Table 61b. continued.

Day 36. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
36a	62.6	67,	75,	77,	80,	19,	19,	21,	22,	3,	3,	70
42	68.8	74,	76,	77,	80,	70,	74,	76,	83,	14,	16,	10
44	48.5	87,	95,	99,	120,	85,	91,	97,	103,	52,	99,	1
46	69.3	90,	94,	97,	105,	65,	75,	100,	102,	73,	100,	1

Day 47. Perfuser drained and refilled with 250 ml. of 100 ppm. MCPA solution. Perfuser re-started, sampled after 1 hr.

47a	73.0	65,	71,	73,	97,	19,	22,	22,	23,	3,	3,	65
52	54.9	44,	53,	69,	71,	66,	67,	67,	80,	24,	29,	5
54	74.3	66,	70,	81,	99,	94,	94,	96,	98,	82,	98,	<1
56	72.9	82,	92,	103,	111,	96,	96,	103,	106,	88,	92,	<1

Day 57. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding to the perfuser and after 1 hr. perfusion.

A.	G.	H.	A.	G.	H.	A.	G.	H.
57ba	100	200,	65	39.5	79,	76	25.5	51,
57alhr	59	118,	66	37.5	75,	78	23	46,
58	52.1	104,	67	/	/,	80	17.5	35,
59	50	100,	68	29.5	59,	82	20	40,
60	46	92,	69	/	/,	84	15.6	31,
61	45.5	91,	70	32	64,	86	11.7	23,
62	45	90,	72	29.6	59,	88	9.5	19,
63	42	84,	74	25.2	50,	90	9.2	18,
64	42	84,	75	26	52,	92	6	12,

Day 94. 25 mls. of 1,000 ppm. MCPA solution added without draining making the perfusate approximately 100 ppm. MCPA. Perfuser continued, sampled after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
94a	75.2	68,	71,	72,	77,	24,	24,	31,	31,	1.5,	2.5,	90
97	75.2	69,	76,	99,	100,	18,	18,	18,	25,	3,	5.5,	80
99	72.9	54,	65,	67,	73,	15,	23,	27,	27,	1.5,	2.5,	80
101	78.6	79,	85,	89,	91,	18,	23,	28,	38,	4,	4,	50
105	64.1	67,	69,	69,	91,	23,	31,	39,	42,	3,	4.5,	40
107	52.7	76,	89,	110,	122,	32,	34,	36,	48,	4,	5.5,	30
109	59.7	103,	103,	117,	167,	45,	48,	62,	73,	8.5,	10,	15
111	61.6	75,	80,	80,	99,	65,	65,	83,	83,	13,	15,	10
113	78.0	85,	91,	95,	98,	84,	87,	92,	119,	118,	122,	<1

Table 62. Direct perfusion of 2,4-dimethylphenoxyacetic acid.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.1 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
E. Longest roots at nominal concentration of 10 ppm. as % m.c.
F. Indicated 2,4-DM concentration in the perfusate (ppm.).

Perfusion started on 25/5/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 10 ppm. 2,4-DM solution. First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	89.8	66,	67,	70,	74,	16,	16,	17,	18,	2,	2,	10.0
20	91.7	51,	53,	57,	58,	17,	21,	22,	29,	4.5,	4.5,	8.0
40	98.6	78,	78,	80,	86,	31,	31,	32,	39,	11,	14,	4.5
60	102.6	88,	92,	99,	101,	37,	38,	42,	47,	12,	15,	1.5
70b	96.1	93,	95,	98,	102,	67,	67,	70,	71,	25,	30,	0.5

Day 70. Perfuser drained and refilled with 250 ml. of 10 ppm. 2,4-DM solution. Perfuser re-started, sampled after 1 hr.

70a	102.7	77,	78,	80,	83,	21,	23,	23,	24,	2,	3,	7.0
80	94.0	77,	77,	78,	82,	26,	27,	31,	32,	2,	3,	5.5
90	97.3	79,	80,	80,	83,	26,	28,	37,	41,	3,	5,	4.0

Table 63a. Direct perfusion of 2,4-dimethylphenoxyacetic acid.

Key to columns in table: as in Table 62, above.

Perfusion started on 25/5/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 100 ppm. 2,4-DM solution. First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	89.8	64,	66,	66,	67,	14,	15,	18,	20,	2,	3.5,	100
20	91.7	50,	54,	56,	71,	18,	19,	19,	20,	2,	3.5,	95
40	98.6	68,	68,	70,	73,	18,	30,	31,	32,	3,	5,	65

Table 62a. Direct perfusion of 2,4-dimethylphenoxyacetic acid.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.1 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
E. Longest roots at nominal concentration of 10 ppm. as % m.c.
F. Indicated 2,4-DM concentration in the perfusate (ppm.).

Perfusion started on 4/2/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 10 ppm. 2,4-DM solution. First sample taken after 1 hr. perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	85.9	47,	48,	51,	69,	16,	17,	19,	33,	1,	2.5,	10.0
10	78.0	45,	50,	56,	60,	13,	18,	19,	21,	2.5,	4,	11.5
20	80.8	48,	54,	58,	59,	16,	17,	19,	22,	1,	2.5	11.0
30	75.9	51,	54,	58,	62,	14,	16,	17,	17,	1.5,	4,	12.5
40	71.0	56,	60,	70,	73,	18,	19,	20,	25,	4,	5.5,	9.0
50b	79.9	41,	43,	46,	51,	16,	17,	20,	20,	4,	5,	9.5

Day 50. Perfuser nearly empty so not drained but refilled with 250 ml. of 100 ppm. 2,4-DM solution. Perfuser re-started, sampled after 1 hr.

50a	79.9	50,	56,	59,	69,	15,	16,	19,	19,	2.5,	3,	95
60	74.7	32,	35,	35,	40,	16,	16,	17,	17,	1.5,	2.5,	105
70	80.7	53,	58,	59,	66,	15,	18,	18,	19,	2,	3.5,	95
75	73.9	55,	60,	61,	68,	20,	20,	22,	24,	4,	6,	85
80	70.7	76,	76,	77,	103,	17,	17,	17,	19,	3,	3,	100
90	80.6	67,	73,	74,	79,	20,	22,	22,	22,	1,	2.5,	80
100	75.1	77,	90,	96,	100,	20,	23,	23,	33,	4,	4,	55
110	89.8	81,	81,	89,	93,	31,	32,	33,	36,	6.5,	9,	40
120	92.3	88,	98,	100,	103,	35,	35,	37,	37,	15,	17,	10

Table 63. Direct perfusion of 2,4-dimethylphenoxyacetic acid.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.1 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
E. Longest roots at nominal concentration of 10 ppm. as % m.c.
F. Indicated 2,4-DM concentration in the perfusate (ppm.).

Perfusion started on 4/2/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 100 ppm. 2,4-DM solution. First sample taken after 1 hr perfusion.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	85.9	62,	63,	67,	72,	14,	14,	20,	21,	3.5,	4.5,	90
10	78.0	37,	37,	40,	40,	16,	18,	18,	24,	1.5,	2.5,	100
20	80.8	36,	37,	37,	43,	17,	21,	27,	29,	1,	2.5,	85
30	75.9	32,	33,	34,	34,	12,	13,	15,	20,	0,	1.5,	115
40	71.0	37,	41,	44,	46,	12,	14,	14,	17,	1.5,	3,	125
50,	79.9	44,	45,	54,	56,	13,	14,	14,	20,	1,	4,	130
60	74.7	51,	53,	57,	65,	12,	16,	18,	19,	1.5,	2.5,	120
70	80.7	53,	55,	60,	65,	15,	18,	25,	27,	2,	3.5,	85
75	73.9	53,	57,	61,	72,	16,	18,	23,	27,	2.5,	4,	80
80	70.7	68,	77,	80,	89,	25,	27,	37,	38,	8.5,	16,	45
85b	75.5	101,	101,	106,	106,	26,	29,	29,	32,	4,	5.5,	50

Day 85. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-DM solution. Perfuser re-started, sampled after 1 hr.

85a	81.4	54,	60,	67,	70,	17,	18,	19,	26,	2.5,	3.5,	95
90	80.6	67,	68,	69,	73,	17,	18,	20,	22,	2.5,	3.5,	75
100	75.1	73,	74,	76,	85,	17,	18,	19,	21,	2.5,	4,	65
110	89.8	82,	92,	95,	96,	22,	23,	25,	25,	4.5,	5.5,	60
120	92.3	89,	89,	92,	99,	37,	37,	38,	39,	4.5,	4.5,	30
130	91.7	96,	100,	102,	102,	33,	41,	44,	50,	6.5,	11,	25
140	100.1	91,	94,	106,	106,	52,	52,	57,	65,	15,	16,	12
150	99.3	93,	94,	94,	95,	54,	54,	57,	59,	27,	32,	13
160	97.3	79,	79,	89,	92,	67,	68,	72,	77,	29,	29,	6
180	102.6	98,	101,	101,	103,	61,	61,	63,	65,	22,	31,	6

Note. The apparent rise in concentration during the first 50 days of perfusion was not due to evaporation of the perfusate. Growth in the nominal 0.1 ppm. dilution showed a relatively greater increase in toxicity than growth in the other two dilutions. This suggests the formation of another toxic compound with a slightly different concentration / toxicity curve, the difference appearing at the 0.1 ppm. dilution but being masked by residual 2,4-DM at the other two concentrations.

Table 64. Direct perfusion of ∝-4-CPP, followed by 4-CPA.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Indicated ∝-4-CPP concentration in the perfusate (ppm.).
G. Indicated 4-CPA concentration in the perfusate (ppm.).

Perfusion started on 28/5/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 100 ppm. ∝-4-CPP solution, (solution common with that of Table 64a,). Solution sampled before adding to perfuser.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	95.0	70,	78,	92,	95,	23,	25,	28,	29,	11,	16,	100
4	93.9	80,	83,	89,	89,	40,	43,	54,	54,	10,	13	100
8	98.3	88,	90,	93,	111,	34,	40,	47,	48,	11,	12,	100
12	95.2	94,	99,	100,	107,	92,	92,	103,	107,	64,	70,	2.5

Day 16. Perfuser drained and refilled with 250 ml. of 100 ppm. ∝-4-CPP solution. Perfuser re-started, sampled after 1 hr.

16a	94.9	86,	86,	87,	91,	31,	34,	35,	37,	7.5,	8.5,	110
20	93.7	87,	88,	95,	100,	92,	97,	98,	105,	60,	71,	2.5

Day 24. Perfuser drained and refilled with 250 ml. of 100 ppm. ∝-4-CPP solution. Perfuser re-started, sampled after 1 hr.

24a	101.7	84,	86,	97,	103,	32,	32,	35,	35,	16,	17,	70
28	100.1	105,	105,	107,	107,	101,	102,	104,	108,	94,	101,	<1

Day 30. Perfuser drained and refilled with 250 ml. of 100 ppm. ∝-4-CPP solution. Perfuser re-started, sampled after 1 hr.

30a	99.8	76,	85,	93,	109,	46,	47,	52,	52,	14,	29,	70
32	102.7	92,	92,	93,	107,	92,	96,	96,	104,	88,	94,	<1

Day 34. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
34a	97.5	85,	86,	88,	97,	25,	26,	29,	32,	4,	4,	75
36	97.5	87,	94,	99,	100,	93,	94,	96,	104,	102,	104,	<1

Day 38. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr.

38	96.6	90,	93,	96,	103,	29,	31,	35,	41,	5,	6,	70

Table 64. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
40b	96.6	100,	104,	104,	104,	99,	100,	108,	118,	100,	104,	<1

Day 40. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr.

40a	96.6	90,	91,	94,	98,	26,	28,	30,	33,	4,	6,	80
42	95.2	100,	100	102,	105,	101,	101,	103,	109,	115,	116,	<1

Day 44. Perfuser drained and refilled with 250 ml. of 100 ppm. ∝-4-CPP solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
44a	98.6	84,	91,	91,	93,	44,	46,	54,	60,	14,	14,	70
46	102.9	95,	97,	100,	102,	92,	93,	99,	101,	62,	89,	2

Table 64a. Direct perfusion of ∝-4-CPP, followed by 4-CPA.

Key to columns in table: as in Table 64, above.

Details of perfusion set-up: as in Table 64, above.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
0	95.0	70,	78,	92,	95,	23,	25,	28,	29,	11,	16,	100
4	93.9	79,	84,	89,	94,	30,	35,	39,	48,	10,	12,	110
8	98.3	77,	82,	87,	88,	26,	29,	30,	31,	11,	14,	100
12	95.2	70,	76,	83,	84,	29,	30,	30,	37,	9.5,	11,	120
16	88.3	81,	81,	87,	95,	82,	84,	86,	86,	43,	70,	40

Day 20. Perfuser drained and refilled with 250 ml. of 100 ppm. ∝-4-CPP solution. Perfuser re-started, sampled after 1 hr.

20a	93.7	57,	67,	68,	71,	22,	22,	25,	28,	9.5,	12,	120
24	104,1	87,	90,	92,	93,	89,	96,	96,	111,	77,	77,	15

Day 28. Perfuser drained and refilled with 250 ml. of 100 ppm. ∝-4-CPP solution. Perfuser re-started, sampled after 1 hr.

28a	100.1	96,	97,	97,	100,	38,	39,	44,	44,	13,	14,	80
30	99.8	97,	105,	107,	112,	70,	75,	83,	83,	28,	33,	12
32b	97.5	93,	95,	96,	98,	103,	105,	107,	110,	89,	96,	<1

Day 32. Perfuser drained and refilled with 250 ml. of 100 ppm. ∝-4-CPP solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
32a	102.7	80,	82,	95,	98,	40,	40,	41,	44,	14,	16,	80
34	101.4	95,	100,	102,	114,	99,	100,	100,	100,	79,	84,	<1

Day 36. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
36a	99.3	89,	91,	104,	115,	30,	31,	35,	37,	6,	7,	65
38	96.6	94,	96,	97,	99,	92,	93,	105,	110,	89,	95,	<1

Day 40. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CPA solution. Perfuser re-started, sampled after 1 hr.

40a	105.4	88,	88,	90,	94,	28,	31,	32,	35,	4.5,	5.5,	70
42	95.2	94,	94,	99,	112,	96,	108,	108,	112,	87,	93,	<1

Day 44. Perfuser drained and refilled with 250 ml. of 100 ppm. α-4-CPP solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	F.
44a.	98.6	88,	92,	94,	98,	38,	38,	39,	40,	12,	14,	90
46	102.9	95,	95,	96,	98,	93,	97,	99,	100,	90,	100,	<1

Table 65. Direct perfusion of ∝-4-CPP, followed by 2,4-D.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Longest roots at nominal concentration of 10 ppm. as % m.c.
G. Indicated ∝-4-CPP concentration in the perfusate (ppm.).
H. Indicated 2,4-D concentration in the perfusate (ppm.).

Perfusion started on 28/7/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 100 ppm. ∝-4-CPP solution. (Solution common with that of Table 66a,). Solution sampled before adding to perfuser.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	G.
0	101.2	44,	45,	48,	58,	15,	16,	19,	20,	3,	3,	100
6	99.4	40,	44,	48,	54,	16,	17,	19,	20,	3,	3,	95
12	102.3	91,	92,	94,	103,	86,	96,	97,	97,	33,	41,	2
15	100.3	105,	106,	107,	109,	90,	92,	98,	102,	38,	41,	2
18b	100.0	97,	98,	101,	112,	90,	98,	99,	101,	38,	44,	2

Day 18. Perfuser drained and refilled with 250 ml. of 100 ppm. ∝-4-CPP solution. Perfuser re-started, sampled after 1 hr.

18a	96.1	45,	46,	52,	55,	11,	12,	13,	15,	2,	2,	110
21	101.4	88,	91,	91,	99,	52,	52,	56,	57,	17,	21,	28
24	96.4	91,	98,	99,	100,	94,	99,	100,	101,	38,	39,	2
27b	95.2	100,	101,	101,	102,	102,	103,	103,	106,	40,	40,	2

Day 27. Perfuser drained and refilled with 250 ml. of 100 ppm. ∝-4-CPP solution. Perfuser re-started, sampled after 1 hr.

27a	100.6	53,	57,	58,	61,	14,	14,	15,	17,	2,	3,	100
30	99.1	89,	90,	92,	101,	87,	90,	91,	91,	41,	42,	2
32b	97.0	96,	96,	98,	103,	94,	94,	100,	101,	38,	40,	2

Day 32. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	H.
32a	97.0	91,	95,	100,	100,	24,	25,	25,	28,	3,	7,	90
36	97.3	97,	101,	101,	110,	42,	43,	48,	49,	9.5,	11,	30

Table 66. Direct perfusion of ∝-4-CPP followed by ∝-2,4-DCPP.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Longest roots at nominal concentration of 10 ppm. as % m.c.
G. Indicated ∝-4-CPP concentration in the perfusate (ppm.).
H. Indicated ∝-2,4-DCPP concentration in the perfusate (ppm.).

Perfusion started on 23/2/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 100 ppm. ∝-4-CPP solution. Solution sampled before adding to perfuser.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	H.
0	84.1	44,	45,	47,	64,	25,	25,	25,	31,	10,	12,	100
3	88.2	50,	55,	55,	64,	22,	23,	24,	27,	9,	12,	120
6	82.0	55,	57,	60,	71,	18,	22,	24,	30,	10,	11,	120
9	86.1	42,	43,	52,	63,	23,	26,	26,	27,	8,	12,	140
12	67.8	65,	69,	71,	96,	24,	25,	28,	32,	7.5,	9,	160
15	91.3	56,	66,	67,	80,	33,	33,	33,	34,	13,	14,	80
18	81.9	82,	82,	87,	94,	67,	68,	74,	79,	67,	71,	2.5
21b	82.7	93,	95,	98,	98,	102,	102,	102,	113,	67,	87,	1.5

Day 21. Perfuser drained and refilled with 250 ml. of 100 ppm. ∝-4-CPP solution. Perfuser re-started, sampled after 1 hr.

21a	82.7	71,	75,	86,	98,	25,	27,	27,	28,	11,	15,	90
24	77.0	92,	96,	99,	106,	82,	86,	97,	100,	74,	75,	1.5
27b	73.9	95,	95,	101,	108,	81,	88,	100,	111,	88,	116,	1

Day 27. Perfuser drained and refilled with 250 ml. of 250 ppm. ∝-4-CPP solution. Perfuser re-started, sampled after 1 hr.

27a	73.9	45,	51,	68,	89,	19,	19,	22,	22,	5.5,	8,	235
30	89.4	95,	96,	97,	107,	66,	72,	77,	89,	51,	59,	4
33b	84.6	73,	77,	80,	85,	65,	80,	101,	104,	44,	67,	4

Day 33. Perfuser drained and refilled with 250 ml. of 500 ppm. ∝-4-CPP solution. Perfuser re-started, sampled after 1 hr.

33a	84.6	30,	33,	34,	35,	15,	15,	16,	16,	2.5,	3.5,	500
36	83.1	29,	30,	31,	36,	15,	17,	19,	24,	6,	9.5	450
39	72.1	33,	33,	39,	46,	16,	18,	20,,	21,	4,	8.5,	350
42	78.2	47,	52,	60,	73,	16,	19,	19,	24,	5,	5,	350
45	83.4	42,	45,	55,	61,	22,	22,	26,	33,	6,	6,	250
48	72.1	70,	70,	73,	90,	61,	68,	73,	79,	30,	33,	20
51	80.7	91,	93,	99,	101,	84,	91,	116,	119,	64,	79,	3

Day 54. Perfuser drained and refilled with 250 ml. of 500 ppm. ∝-4-CPP solution. Perfuser re-started, sampled after 1 hr.

Table 66. continued.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
54a	74.9	36,	39,	40,	43,	21,	23,	23,	24,	4,	5.5,	350
57	82.1	39,	41,	49,	51,	21,	21,	24,	26,	3.5,	5,	300
60	70.7	106,	109,	110,	119,	100,	102,	110,	117,	47,	51,	5
63	69.9	106,	109,	114,	126,	99,	103,	109,	110,	104,	117,	<1
66b	71.0	82,	94,	97,	111,	83,	94,	106,	121,	80,	92,	<1

Day 66. Perfuser drained and refilled with 250 ml. of 1,000 ppm. ∝-4-CPP solution. Perfuser re-started, sampled after 1 hr.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	G.
66a	71.0	27,	34,	34,	35,	11,	12,	12,	13,	1.5,	3,	1,000
69	75.5	24,	24,	28,	40,	13,	13,	15,	16,	1.5,	2.5,	900
72	83.9	44,	45,	54,	56,	12,	12,	15,	21,	2.5,	2.5,	650
75	84.3	34,	37,	38,	47,	15,	18,	19,	20,	2.5,	2.5,	450
78	80.7	68,	68,	79,	82,	25,	25,	27,	29,	11,	14,	200
81	75.1	73,	73,	82,	101,	40,	40,	43,	52,	9.5,	14,	100
84	80.8	74,	79,	82,	83,	30,	31,	38,	51,	8.5,	11,	120
87	85.3	63,	64,	70,	75,	29,	29,	35,	43,	16,	26,	100
90	89.8	62,	66,	78,	93,	29,	29,	30,	32,	12,	12,	100
93	95.0	76,	84,	86,	89,	32,	32,	35,	41,	9.5,	13,	120
96	93.3	89,	90,	94,	98,	33,	35,	35,	36,	9.5,	14,	110
99	93.9	77,	83,	87,	104,	33,	33,	34,	42,	11,	14,	120
102	98.3	76,	78,	79,	85,	31,	34,	36,	53,	11,	15,	120
105	100.5	79,	82,	84,	88,	39,	43,	51,	52,	11,	12,	100
108b	88.7	66,	72,	72,	102,	29,	29,	37,	43,	13,	14,	90

Day 108. Perfuser drained and refilled with 250 ml. of 1,000 ppm. ∝-4-CPP solution. Perfuser re-started, sampled after 1 hr.

108a	88.7	34,	38,	39,	56,	9,	11,	13,	14,	1,	2,	1,000
114	93.7	50,	55,	62,	76,	12,	16,	16,	17,	3,	4.5,	500
120	98.1	82,	86,	92,	100,	21,	21,	26,	27,	8,	12,	200
126	102.7	86,	95,	98,	99,	69,	75,	80,	98,	30,	32,	15
132	96.6	95,	96,	97,	116,	82,	88,	88,	99,	70,	94,	1

Day 138. Perfuser drained and refilled with 250 ml. of 100 ppm. ∝-2,4-DCPP solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	H.
138a	98.6	77,	78,	80,	84,	26,	27,	27,	28,	5,	5,	110
144	95.9	101,	105,	108,	111,	79,	83,	98,	108,	20,	25,	10
147	98.4	99,	99,	103,	103,	91,	96,	98,	99,	56,	61,	4
150b	99.7	86,	93,	99,	102,	93,	94,	95,	95,	78,	81,	1.5

Day 150. Perfuser drained and refilled with 250 ml. of 100 ppm. ∝-2,4-DCPP solution. Perfuser re-started, sampled after 1 hr.

Table 66. continued.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	H.
150a	99.7	30,	36,	37,	43,	6,	6,	7,	9,	1,	2,	80
153	99.8	36,	37,	40,	41,	9,	11,	12,	12,	1,	2,	45
156	101.2	54,	58,	60,	67,	19,	21,	23,	25,	3,	3,	20
159	98.4	93,	93,	94,	96,	52,	53,	54,	71,	12,	13,	4.5
162	99.4	93,	93,	93,	104,	93,	96,	100,	112,	56,	64,	3
165	102.3	99,	101,	103,	107,	95,	104,	108,	118,	83,	89,	1

Note. Between days 78 and 108, complete breakdown of the more active isomer may be inhibited by a high residual concentration of inactive, résistant isomer.

Table 66a. Direct perfusion of ∝-4-CPP followed by ∝-2,4-DCPP.

Key to columns in table: as in Table 66, above.

Perfusion started on 28/7/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 100 ppm. ∝-4-CPP solution. Solution sampled before adding to perfuser.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	G.
0	101.2	44,	45,	48,	58,	15,	16,	19,	20,	3,	3,	100
6	99.4	37,	39,	39,	55,	15,	17,	17,	18,	3,	3,	100
12	102.3	34,	37,	38,	47,	20,	20,	21,	21,	2,	3,	100
15	100.3	52,	55,	58,	59,	10,	12,	12,	15,	2,	3,	65
18	96.1	93,	97,	97,	99,	85,	88,	92,	102,	25,	33,	3.5
21b	101.4	97,	103,	105,	107,	91,	92,	99,	103,	32,	32,	2.5

Day 21. Perfuser drained and refilled with 250 ml. of 100 ppm. ∝-4-CPP solution. Perfuser re-started, sampled after 1 hr.

21a	96.3	49,	50,	51,	60,	11,	12,	12,	13,	3,	3,	105
24	96.4	74,	83,	84,	89,	23,	24,	24,	27,	8.5,	9.5,	35
27b	95.2	97,	98,	100,	103,	100,	100,	101,	103,	46,	52,	1

Day 27. Perfuser drained and refilled with 250 ml. of 100 ppm. ∝-4-CPP solution. Perfuser re-started, sampled after 1 hr.

27a	100.6	50,	52,	53,	66,	13,	16,	16,	20,	2,	3,	95
30	99.1	98,	99,	100,	104,	90,	95,	96,	97,	35,	36,	2
32b	97.0	98,	98,	99,	100,	94,	95,	96,	96,	34,	34,	2

Day 32. Perfuser drained and refilled with 250 ml. of 100 ppm. ∝-2,4-DCPP solution. Perfuser re-started, sampled after 1 hr.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	H.
32a	97.0	28,	33,	34,	36,	5,	5,	7,	9,	1,	2,	95
36	97.3	29,	31,	38,	39,	6,	7,	7,	9,	2,	3,	85

Table 67. Direct perfusion of ∝-2,4-DCPP, followed by 2,4-D.

Key to columns in table:
A. Day of perfusion.
B. Mean control root length (mm.).
C. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage mean control.
D. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
E. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
F. Longest roots at nominal concentration of 10 ppm. as % m.c.
G. Indicated ∝-2,4-DCPP concentration in the perfusate (ppm.).
H. Indicated 2,4-D concentration in the perfusate (ppm.).

Perfusion started on 25/5/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 100 ppm. ∝-2,4-DCPP solution. First sample after 1 hr. perfusion.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	G.
0	89.8	21,	22,	24,	27,	4.5,	5.5,	6.5,	8,	0,	1,	90
20	91.7	22,	29,	31,	35,	3.5,	3.5,	4.5,	6.5,	0.5,	1,	100
40	98.6	28,	29,	33,	34,	5,	6,	6,	7,	1,	2,	90
60	99.8	88,	92,	93,	98,	35,	36,	37,	38,	9,	9,	5
70	99.4	90,	90,	92,	98,	43,	50,	51,	57,	10,	11,	4
75b	97.5	82,	87,	94,	100,	28,	29,	34,	36,	9,	9,	5

Day 75. Perfuser drained and refilled with 250 ml. of 100 ppm. ∝-2,4-DCPP solution. Perfuser re-started, sampled after 1 hr.

75a	97.5	28,	30,	35,	43,	5,	6,	6,	8,	1,	1,	80
80	97.5	28,	32,	34,	36,	6,	6,	7,	8,	1,	2,	80
85	101.4	52,	54,	56,	56,	15,	16,	18,	19,	3,	5,	20
90	94.0	88,	91,	94,	96,	34,	37,	37,	43,	4.5,	5.5,	8
95b	96.1	92,	97,	98,	101,	36,	41,	43,	45,	14,	15,	3

Day 95. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	H.
95a	97.0	90,	91,	93,	93,	30,	31,	35,	37,	5,	7,	90
100	97.3	95,	110,	110,	115,	96,	97,	102,	104,	71,	72,	2.5

Table 67a. Direct perfusion of ∝-2,4-DCPP.

Key to columns in table: as in Table 67, above.

Details of perfusion set-up: as in Table 67, above, except that 250 ml. of 10 ppm. ∝-2,4-DCPP were used.

Table 67a. continued.

A.	B.	D.	D.	D.	D.	E.	E.	E.	E.	F.	F.	G.
0	89.8	19,	21,	22,	26,	3.5,	4.5,	4.5,	4.5,	0,	1,	14.0
20	91.7	21,	21,	23,	24,	4.5,	4.5,	5.5,	6.5,	0.5,	0.5,	10.0
40	98.6	23,	23,	25,	25,	4,	4,	5,	6,	1,	1,	11.0
60	99.8	23,	23,	23,	32,	3,	4,	4,	5,	0,	1,	14.0
70	99.4	25,	25,	31,	40,	7,	8,	8,	10,	1,	2,	7.0
Day 80. Perfuser drained and refilled with 250 ml. of 10 ppm. α-2,4-DCPP solution. Perfuser re-started, sampled after 1 hr.												
80a	101.4	29,	31,	35,	37,	6,	6,	7,	8,	1,	2,	8.0
85	101.4	32,	35,	36,	37,	8,	9,	9,	11,	2,	2,	6.0
90	94.0	24,	26,	28,	31,	7.5,	7.5,	7.5,	8.5,	1,	1,	7.0
100	97.3	27,	30,	35,	36,	6,	7,	8,	12,	1,	2,	6.5

Table 67b. Direct perfusion of α-2,4-DCPP.

Key to columns in table: as in Table 67, above.

Perfusion started on 24/2/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 200 ppm. α-2,4-DCPP solution. Solution sampled before adding to perfuser.

A.	B.	C.	C.	C.	C.	D.	D.	D.	D.	E.	E.	G.
0	80.8	40,	62,	67,	78,	15,	16,	18,	18,	2,	4.5,	200
4	86.1	39,	46,	48,	61,	15,	17,	18,	20,	3.5,	4.5,	200
8	86.1	41,	45,	50,	59,	19,	22,	22,	23,	3.5,	3.5,	160
12	76.0	50,	53,	63,	85,	17,	18,	21,	22,	2.5,	4,	180
16	88.4	71,	72,	72,	85,	17,	18,	18,	26,	4.5,	7,	190
20	72.8	48,	51,	63,	71,	18,	22,	22,	23,	3,	4,	150
24	71.1	39,	45,	48,	53,	21,	21,	22,	31,	3,	3,	150
28	76.7	61,	62,	70,	79,	19,	20,	22,	25,	4,	5,	160
32	84.6	44,	45,	50,	56,	16,	17,	17,	19,	2.5,	4.5,	200
36	82.5	33,	34,	38,	40,	16,	17,	17,	22,	3.5,	5,	190
40	74.7	45,	47,	63,	72,	18,	19,	24,	24,	4,	4,	160
44	83.4	35,	38,	39,	43,	18,	18,	19,	20,	2.5,	3.5,	190
48	77.9	51,	51,	64,	64,	18,	19,	20,	22,	5,	7.5,	160
52	81.7	39,	42,	44,	60,	13,	14,	15,	15,	3.5,	3.5,	250
56	82.1	49,	57,	61,	66,	19,	20,	21,	28,	3.5,	5,	160
60	70.7	59,	59,	61,	69,	15,	17,	17,	19,	3,	4,	190
64	81.9	61,	67,	76,	89,	21,	22,	22,	24,	1,	2.5,	140
68	81.4	75,	80,	87,	90,	24,	28,	38,	39,	6,	7.5,	100
72	83.9	87,	88,	91,	94,	45,	46,	52,	54,	13,	18,	50
76	88.6	93,	95,	96,	103,	72,	74,	82,	82,	18,	23,	16
80	75.1	110,	112,	122,	126,	90,	92,	98,	124,	37,	48,	6

Table 67b. continued.

A.	B.	C. C. C. C.	D. D. D. D.	E. E.	G.
84	87.6	98,103,107,109,	79, 82, 85, 92,	21, 25,	13
88	88.3	93, 96,100,100,	79, 79, 84, 89,	22, 24,	13
91	92.6	93, 94, 96, 99,	80, 84, 84, 85,	31, 33,	11
94b	93.3	89, 92, 93, 94,	74, 77, 83, 89,	20, 24,	13

Day 94. Perfuser drained and refilled with 250 ml. of 100 ppm. ∝-2,4-DCPP solution. Perfuser re-started, sampled after 1 hr.

A.	B.	D. D. D. D.	E. E. E. E.	F. F.	G.
94a	93.3	20, 22, 22, 26,	5.5,5.5,6.5,6.5,	0.5,0.5,	100
97	93.9	20, 29, 30, 36,	3, 4, 4,5.5,	1, 1,	100
100	92.3	24, 25, 27, 36,	4.5,5.5,5.5,5.5,	0.5,0.5,	105
103	98.6	30, 31, 39, 40,	5, 6, 6, 6,	0.5,0.5,	90
109	88.3	52, 61, 63, 68,	19, 19, 20, 24,	1, 2,	20
115	94.4	87, 92, 98,108,	41, 42, 45, 54,	9.5, 16,	5
118	101.7	75, 82, 86, 98,	55, 56, 59, 67,	7, 10,	5
121	100.1	99,101,105,112,	43, 51, 55, 58,	8, 9,	5
124	102.9	87, 91, 95, 95,	39, 39, 41, 52,	10, 13,	6
127	101.4	93, 93, 94, 95,	51, 52, 59, 59,	10, 13,	5
130	99.3	90, 92, 93,111,	34, 35, 38, 42,	10, 13,	6
133	96.6	95, 96,103,107,	44, 44, 45, 51,	8.5, 11,	5
136b	99.9	91, 91, 92, 97,	43, 48, 48, 58,	10, 10,	5

Day 136. Perfuser drained and refilled with 250 ml. of 1,000 ppm. ∝-2,4-DCPP solution. Perfuser re-started, sampled after 1 hr.

136a	99.9	26, 28, 28, 32,	6, 8, 8, 10,	2, 2,	1,000
139	102.9	29, 31, 34, 36,	5, 6, 6, 8,	1, 2,	900
142	99.6	27, 27, 28, 32,	5, 5, 5, 6,	1, 1,	1,000
148	102.6	27, 29, 29, 39,	5, 5, 6, 7,	1, 2,	1,000
154	101.2	23, 24, 26, 28,	6, 7, 8, 9,	1, 1,	850
160	99.4	25, 27, 30, 34,	6, 6, 7, 7,	1, 2,	1,000
166	102.3	31, 31, 32, 41,	6, 7, 8, 11,	1, 2,	900
172	95.8	27, 28, 29, 36,	6.5,7.5,8.5, 13,	1, 2,	1,000
184	99.1	25, 27, 29, 36,	4, 5, 5, 6,	1, 2,	1,000

Table 68. Direct perfusion of 4-chlorphenol.

Key to columns in table:
A. Day of perfusion.
B. Colourimeter reading (E.E.L.) in divisions.
C. Indicated 4-CP concentration in the perfusate (ppm.).

Perfusion started on 7/7/52 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried February 1952,) and 250 ml. of 100 ppm. 4-CP solution. Solution (common with Tables 68a, 68b, 68c,) sampled before adding to the perfuser and after 1 hr. perfusion.

A.	B.	C.	A.	B.	C.	A.	B.	C.
0ba	80	106,	11	11.5	15,	30	7.7	10,
0alhr	63	84,	12	12.2	16,	31	9.7	13,
1	50.4	67,	13	13.1	17,	32	6.7	9,
2	43.7	58,	14	13	17,	33	8.7	12,
3	34.1	45,	15	13.3	18,	34	7.6	10,
4	27.8	37,	16	11.8	16,	35	8.1	11,
5	24.2	32,	17	11.3	15,	36	7.3	9,
6	24.4	32,	18	11	15,	37	7.5	10,
7	22.6	30,	19	12.2	16,	38	8.1	11,
8	21.0	28,	20	12.2	16,	39,	7.5	10,
9	17.2	23,	21	10.2	14	40b	9.5	13,
10	15.1	20,	22	11.6	15,			

Day 40. Approximately 25 ml. of 1,000 ppm. 4-CP solution and a little water added to make the perfusate approximately 250 ml. of 100 ppm. 4-CP solution. Sampled after 1 hr. perfusion.

40alhr	58.3	77,	43	6.8	9,	46b	7.2	9,
41	48	64,	44	6.3	8,			
42	20	27,	45	6.5	9,			

Day 46. Perfuser drained and refilled with 250 ml. of approx. 100 ppm. 4-CP solution. Solution sampled before adding to perfuser and after 1 hr. perfusion.

46ba	65	86,	48	23.7	31,	51b	4.6	6,
46alhr	48.1	64,	49	11	15,			
47	42.2	56,	50	3.5	4,			

Day 51. Perfuser refilled as on Day 46.

51ba	73	97,	52	28.8	38,	53b	7.2	9,
51alhr	53.2	71,						

Day 53. Perfuser refilled as on Day 46.

53ba	75.5	100,	54	28.7	38,	55b	15.3	20,
53alhr	57	76,						

Day 55. Perfuser refilled as on Day 46.

55ba	74	98,	56	33	44,	57b	12.6	17,
55alhr	54	72,						

Table 68. continued.

Day 57. Perfuser refilled as on Day 46.

A.	B.	C.	A.	B.	C.	A.	B.	C.
57ba	75	100,	57alhr	56.5	75,	58b	32	43,

Day 58. 30 ml. of 1% sodium azide solution added to the perfuser.

58alhr	34.6	46,	59	15	20,	60b	1.4	2,

Day 60. Approximately 20 ml. of 1,000 ppm. 4-CP solution added to the perfuser without draining. Sampled after 1 hr. perfusion.

60alhr	62	85,	62	18.4	24,	64	2.7	4,
61	36	48,	63	6.5	8,	65b	1.2	2,

Day 65. Perfuser refilled as on Day 46.

65ba	71	94,	67	43.2	57,	70	11.2	15,
65alhr	56.5	75,	68	34.8	46,	71b	5	7,
66	48.8	65,	69	19.2	25			

Day 71. Perfuser refilled as on Day 46.

71ba	84.5	112,	71alhr	65.5	87,	72b	45	60,

Day 72. 0.3 gm. of sodium azide dissolved in a little perfusate and added to the perfuser. Sampled after 1 hr.

72alhr	47.5	63,	75	28.6	38,	78	4.5	6,
73	45	60,	76	19.2	26,	79b	2.6	3,
74	35	46,	77	12.8	17,			

Day 79. Perfuser refilled as on Day 46.

79ba	81.5	108,	80	50.4	67,	81b	42.1	56,
79alhr	60	80,						

Day 81. 3.0 gm. of sodium azide dissolved in a little perfusate and added to the perfuser. Sampled after 1 hr.

81alhr	55.5	74,	86	52.7	70,	91	53	70,
82	54	72,	87	53.2	71,	92	53.7	71,
83	56	74	88	52	69,	93	54	72,
84	54	72	89	54	72,	94	53.2	71,
85	55	73,	90	55	73,			

Table 68a. Direct perfusion of 4-chlorphenol.

Key to columns in table: as in Table 68, above.

Details of perfusion set-up: as in Table 68, above. Starting solution common with Tables 68, 68b, 68c.

Table 68a. continued.

A.	B.	C.	A.	B.	C.	A.	B.	C.
0ba	80	107,	5	21.8	29,	11	14.8	20,
0alhr	64	85,	6	23	31,	12	13.7	18,
1	49	65,	7	18.1	24,	13	13	17,
2	44	59,	8	19.7	26,	14b	13	17,
3	34.2	45,	9	19	25,			
4	27.2	36,	10	18.2	24,			

Day 14. 10 ml. of 1,000 ppm. 4-CP solution added to the perfuser without draining, making the perfusate approximately 100 ppm. 4-CP. Sampled after 1 hr. perfusion.

14alhr	59	79,	18	13.7	18,	22	13.6	18,
15	55	73,	19	13.9	18,	30b	10.2	14,
16	43.4	58,	20	14.4	19,			
17	26.6	35,	21	9.6	13,			

Day 30. Approximately 50 ml. of diluted stock 4-CP solution added to the perfuser without draining, making the perfusate approximately 250 ml. of 100 ppm. 4-CP. Sampled after 1 hr.

30alhr	82	108,	36	9.4	13,	42	12.8	17,
31	75.5	101,	37	8.8	12,	43	12.2	16,
32	66	88,	38	10.5	14,	44	11	15,
33	54	72,	39	12	16,	45	11.8	16,
34	22.2	29,	40	12	16,	46	11.2	15,
35	12.5	17,	41	12.8	17,			

Table 68b. Direct perfusion of 4-chlorphenol.

Key to columns in table: as in Table 68, above.

Details of perfusion set-up: as in Table 68, above. Starting solution common with Tables 68, 68a, and 68c.

A.	B.	C.	A.	B.	C.	A.	B.	C.
0ba	80	107,	5	20.6	28,	11	12	16,
0alhr	64	85,	6	18	24,	12	11.7	16,
1	49.7	66,	7	15.8	21,	13	12	16,
2	41	55,	8	15.6	21,	14b	13	17,
3	30	40,	9	13	17,			
4	22.6	31,	10	13.2	18,			

Day 14. Perfuser drained and refilled with 200 ml. of approximately 125 ppm. 4-CP solution, making the perfusate approximately 250 ml. of 100 ppm. 4-CP. Sampled after 1 hr.

Table 68b. continued.

A.	B.	C.	A.	B.	C.	A.	B.	C.
14alhr.	67	89,	16	47.2	63,	18	8.2	11,
15	59.4	79,	17	29.1	39,	19b	8.1	11,

Day 19. Perfuser drained and refilled with 250 ml. of approximately 100 ppm. 4-CP solution. Solution sampled before adding to the perfuser and after 1 hr. perfusion.

19ba	80	107,	20	25	33,	22	5.9	8,
19alhr	60	80,	21	6	8,	30	4.8	6,

Day 30. Perfuser drained and refilled with 250 ml. of 4-CP solution making the final concentration about 100 ppm. in the perfusate. Solution sampled before adding to the perfuser and after 1 hr. perfusion.

30ba	83	111,	35	53	70,	41	14	19,
30alhr	69	92,	36	51.5	69,	42	14.1	19,
31	60.3	80,	37	45	60,	43	13.3	18,
32	56.3	75,	38	45	60,	44	12.9	17,
33	53.5	71,	39	32.8	44,	45	13.3	18,
34	56	74,	40	14	19,	46	11.2	15,

Table 68c. Direct perfusion of 4-chlorphenol.

Key to columns in table: as in Table 68, above.

Details of perfusion set-up: as in Table 68, above. Starting solution common with Tables 68, 68a and 68b.

A.	B.	C.	A.	B.	C.	A.	B.	C.
0ba	80	107,	6	24.1	32,	13	12.5	17,
0alhr	64	85,	7	23	31,	14	11.4	15,
1	50.3	67,	8	21.5	29,	15	13	17,
2	45	60,	9	21	28,	16	12.8	17,
3	35.2	47,	10	18.3	24,	17	11.6	15,
4	29	39,	11	14.7	20,	18	11	15,
5	26.6	35,	12	12.9	17,	19b	10.5	14,

Day 19. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CP solution. Solution sampled before adding to the perfuser and after 1 hr. perfusion.

19ba	80	107,	20	49.5	66,	22	31	41,
19alhr	59.3	84,	21	43	57,	30b	6.2	8,

Table 68c. continued.

Day 30. Perfuser drained and refilled with 250 ml. of 100 ppm. 4-CP. Solution sampled before adding to the perfuser and after 1 hr. perfusion.

A.	B.	C.	A.	B.	C.	A.	B.	C.
30ba	83	111,	31	47.5	63,	32b	34.8	46,
30alhr	57	76,						

Day 32. 25 ml. of 1% sodium azide solution added to the perfuser without draining giving a final concentration of approximately 0.1 %. Perfusate sampled after 1 hr.

32alhr	37.2	49,	37	24	32,	42	21	28,
33	32	43,	38	25.1	33,	43	18.6	25,
34	30.3	40,	39	24.7	33,	44	16.4	22,
35	27.2	36,	40	24	32,	45	15.1	20,
36	26.7	35,	41	23	31,	46	13	17,

Table 69. Attempted transfer of 2,4-DCP adaptation to fresh soil.

Key to columns in table:
A. Day of perfusion.
B. Colourimeter reading (E.E.L. instrument,) in divisions.
C. Indicated 2,4-DCP concentration in the perfusate (ppm.).

Perfusion started on 7/8/51 with 50 gm. of soil (1 to 4 mm., Sussex Lodge soil, dried June 1951,) and 250 ml. of 200 ppm. 2,4-DCP solution, prepared from the complete perfusate drained from a 2,4-DCP enriched perfuser (Table 72b,) on 6/8/51. Solution sampled before adding to perfuser and after 1 hr.

A.	B.	C.	A.	B.	C.	A.	B.	C.
0ba.	112	224,	7	56.5	113,	15	53	106,
0alhr	90	180,	8	52.5	105,	16	50.4	101,
1	67	134,	9	54	108,	17	51	102,
2	64	128,	10	55	110,	18	50	100,
3	61.8	124,	11	48.3	97,	19	41.5	83,
4	60	120,	12	57	114,	20	48	96,
5	53.5	107,	13	54.5	109,	21b	47	94,
6	54	108,	14	50	100,			

Day 21. Perfuser had shown no sign of transferred adaptation so it was dismantled. Perfusate first drained into a sterile flask and used to prepare 250 ml. of approximately 200 ppm. 2,4-DCP solution. This solution used to start a fresh perfuser with 50 gm. of soil as above. Solution sampled before adding and after 1 hr. perfusion.

21ba.	120	240,	32	61	122,	44	47	94,
21alhr	78	156,	33	61.5	123,	45	51	102,
22	72	144,	34	58.5	117,	46	48.9	98,
23	67	134,	35	53	106,	47	51	102,
24	62.5	125,	36	57	114,	48	49.2	98,
25	60	120,	37	58	116,	49	48.5	97,
26	65	130,	38	56	112,	50	49.6	99,
27	66	132,	39	44.5	89,	51	39.8	80,
28	48.8	98,	40	64	128,	52	42.5	85,
29	49	98,	41	52.5	105,	53	37.5	75,
30	63	126,	42	50.5	101,	54	30.3	61,
31	62	124,	43	42	84,	55b	11.2	22,
						This result checked.		

Day 55. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr perfusion.

55ba.	107	214,	60	59.2	118,	66	53.5	107,
55alhr	69	138,	61	65.5	131,	67	57	114,
56	64	128,	62	59.5	119,	68	55.5	111,
57	61	122,	63	57.5	115,	69	53.2	106,
58	64.5	129,	64	57.5	115,	70	53	106,
59	65	130,	65	44	88,	71	53	106,

Table 69. continued.

A.	B.	C.	A.	B.	C.	A.	B.	C.
72	/	/	75	45	90,	78	/	/
73	50	100,	76	/	/	79	21.6	43,
74	/	/	77	36.1	72,	80b	12.9	26,

Day 80. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Sampled before adding to the perfuser and after 1 hr. perfusion.

80ba.	95	190,	90	29.7	59,	99	16	32,
80alhr	69	138,	92	27	54,	100	16	32,
81	53.6	107,	94	22	44,	101	12.1	24,
82	50	100,	95	21	42,	102,	12.1	24,
84	43.6	87,	96	20.8	42,	103,	9.4	19,
86	38.8	78,	97	20.5	41,	104b	5.1	10,
88	37	74,	98	18	36,			

Day 104. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding to the perfuser and after 1 hr.perfusion.

104ba.	88	176,	114	41.2	82,	125	23	46,
104alhr.	71	142,	115	38.3	77,	126	20.2	40,
105	63	126,	116	36.8	74,	127	20	40,
106	57.5	115,	117	35	70,	128	16.8	34,
107	54	108,	118	32	64,	129	17.1	34,
108	49.5	99,	119	27	54,	130	17.5	35,
109	49	98,	120	29.4	59,	131	15.8	32,
110	43.5	87,	121	29.4	59,	132	12.6	25,
111	42.5	85,	122	25	50,	133	10	20,
112	42.8	86,	123	28	56,			
113	41.4	83,	124	24	48,			

Table 70. Direct perfusion of 2,4-DCP followed by 2-CP.

Key to columns in table:
A. Day of perfusion.
B. Colourimeter reading (E.E.L. instrument,) in divisions.
C. Indicated 2,4-DCP concentration in the perfusate (ppm.).
D. Indicated 2-CP concentration in the perfusate (ppm.).

Perfusion started on 19/12/51 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried June 1951,) and 250 ml. of approx. 20 ppm. 2,4-DCP solution. Solution sampled before adding to the perfuser and after 1 hr.

A.	B.	C.	A.	B.	C.	A.	B.	C.
0ba.	12.1	24,	11	5.5	11,	15	6	12,
0alhr.	11	22,	12	3.7	7,	16	4.5	9,
1	10.5	21,	13	4.1	8,	17	4.8	10,
2	8.2	16,	14	5	10,	18b.	4.8	10,
Day 18. Drained and refilled with 250 ml. of 20 ppm. 2,4-DCP.								
18ba.	10.4	21,	19	4.5	9,			
18alhr.	6.3	12,	20b.	1	2,			
Day 20. Drained and refilled with 250 ml. of 25 ppm. 2,4-DCP.								
20ba.	13.1	26,	20alhr.	6.8	14,	21b.	0.5	1,
Day 21. Drained and refilled with 250 ml. of 30 ppm. 2,4-DCP.								
21ba.	14.6	29,	21alhr.	8.6	17,	22b.	0	0,
Day 22. Drained and refilled with 250 ml. of 35 ppm. 2,4-DCP.								
22ba.	18	36,	22alhr.	10	20,	23b.	0	0,
Day 23. Drained and refilled with 250 ml. of 40 ppm. 2,4-DCP.								
23ba.	22	44,	23alhr.	12	24,	24b.	1.8	4,
Day 24. Drained and refilled with 250 ml. of 50 ppm. 2,4-DCP.								
24ba.	27.3	55,	24alhr.	15	30,	25b.	7.6	15,
Day 25. Drained and refilled with 250 ml. of 80 ppm. 2,4-DCP.								
25ba.	39.4	79,	26	20.	40,	28b.	7.6	15,
25alhr.	27.4	55,	27	13	26,			
Day 28. Drained and refilled with 250 ml. of 100 ppm. 2,4-DCP.								
28ba.	49	98,	29	23	46,	31	15	30,
28alhr.	34	68,	30	19	38,	32b.	6.5	13,
Day 32. Drained and refilled with 250 ml. of 100 ppm. 2,4-DCP.								
32ba.	53	106,	33	23.2	46,			
32alhr.	33.7	67,	34b.	11.9	24,			
Day 34. Drained and refilled with 250 ml. of 130 ppm. 2,4-DCP.								
34ba.	66	132,	35	30.1	60,			
34alhr.	38	76,	36b.	10.2	20,			

Table 70. continued.

A.	B.	C.	A.	B.	C.	A.	B.	C.
Day 36. Drained and refilled with 250 ml. of 175 ppm. 2,4-DCP.								
36ba.	87	174,	37	27	54,			
36alhr.	49	98,	38b.	13.4	27,			
Day 38. Drained and refilled with 250 ml. of 200 ppm. 2,4-DCP.								
38ba.	100.5	201,	40	47	94,	43	23	46,
38alhr.	75.5	151,	41	39	78,	44	13.6	27,
39	57.5	115,	42	33	66,	45b	5	10,
Day 45. Drained and refilled with 250 ml. of 200 ppm. 2,4-DCP.								
45ba.	100	200,	47	41	82,	50	20.8	42,
45alhr.	63	126,	48	34.5	69,	51	11.4	23,
46	50.5	101,	49	31.6	63,	52b.	4	8,
Day 52. Drained and refilled with 250 ml. of 200 ppm. 2,4-DCP.								
52ba.	103,	206,	55	43.8	88,	59	19.7	39,
52alhr.	74	148,	56	37.3	75,	60b.	11.6	23,
53	55.5	111,	57	33	66,			
54	47.8	96,	58	27	54,			
Day 60. Drained and refilled with 250 ml. of 200 ppm. 2,4-DCP.								
60ba.	104	208,	64	41	82,	69	20.6	41,
60alhr.	66.5	133,	65	39.7	79,	70	13.4	27,
61	57	114,	66	30.3	61,	71	8.0	16,
62	55	110,	67	29.4	59,	72b.	5.5	11,
63	46.8	94,	68	23.7	47,			
Day 72. Drained and refilled with 250 ml. of 200 ppm. 2,4-DCP.								
72ba.	89	178,	75	37.1	74,	79	12.3	25,
72alhr.	55.5	111,	76	30.2	60,	80b	6.9	14,
73	46	92,	77	20.4	41,			
74	42.3	85,	78	14.7	29,			
Day 80. Drained and refilled with 250 ml. of 200 ppm. 2,4-DCP.								
80ba.	97	194,	84	36.8	74,	89	17	34,
80alhr.	68	136,	85	37.5	75,	90	13.7	27,
81	47.5	95,	86	28	56,	91	11	22,
82	49.5	99,	87	26.8	54,	92b.	5	10,
83	40	80,	88	27	54,			
Day 92. Drained and refilled with 250 ml. of 100 ppm. 2,4-DCP.								
92ba.	50	100,	94	/	/,	97b.	7.2	14,
92alhr.	30	60,	95	11.2	22,			
93	24.5	49,	96	7.4	15,			
Day 97. Drained and refilled with 250 ml. of 100 ppm. 2,4-DCP.								
97ba.	54	108,	99	22.7	45,	102	12.7	25,
97alhr.	31	62,	100	19	38,	103b.	8.5	17,
98	27.6	55,	101	15	30,			

Table 70. continued.

Day.103. Drained and refilled with 250 ml. of 100 ppm. 2-CP.

A.	B.	D.	A.	B.	D.	A.	B.	D.
103ba.	80.5	88,	107	34.5	38,	112	6.2	7,
103alhr.	68	74,	108	24	26,	130	4.5	5,
104	55	60,	109	20.5	22,	131b.	4	4,
105	51	56,	110	12.5	13,			
106	40	44,	111	8	9,			

Day 131. Drained and refilled with 250 ml. of 100 ppm. 2-CP.

131ba.	87	95,	136	24.2	27,	142	14.2	16,
131alhr.	65.5	72,	137	21.8	24,	143	12.3	13,
132	50.5	55,	138	20.5	23,	144	11.2	12,
133	40.7	45,	139	19.6	21,	145	10	11,
134	34.7	38,	140	17	19,	146	7.3	8,
135	32.5	36,	141	14.2	16,	147b.	7.2	8,

Day 147. Drained and refilled with 250 ml. of 100 ppm. 2-CP.

147ba.	87	95,	149	42.5	46,	152	11.6	13,
147alhr.	70	77,	150	35.5	39,	153b.	3.4	4,
148	57	62,	151	25	27,			

Day 153. Drained and refilled with 250 ml. of 100 ppm. 2-CP.

153ba.	88	96,	155	22.2	24,
153alhr.	67	73,	156b.	8.1	9,
154	42.4	46,			

Day 154. Drained and refilled with 250 ml. of 100 ppm. 2-CP.

156ba.	90	99,	157	45	49,	159	21	23,
156alhr.	72.8	80,	158	28.5	31,	160b.	17	19,

Day 160. Drained and refilled with 250 ml. of 100 ppm. 2-CP.

160ba.	89.5	98,	163	33	36,	167	9.6	11,
160alhr.	77.5	85,	164	26.2	29,	168b.	6	7,
161	55	60,	165	19.6	21,			
162	40.3	44,	166	14.2	16,			

Day 168. Drained and refilled with 250 ml. of 100 ppm. 2-CP.

168ba.	92	100,	171	37.8	42,	175	15.6	17,
168alhr.	72.5	80,	172	28.6	31,	176	12.8	14,
169	52	57,	173	24.3	27,	177b.	7.2	8,
170	46.7	51,	174	21.5	24,			

Day 177. Drained and refilled with 250 ml. of 100 ppm. of 2-CP.

177ba.	84.5	92,	179	36.2	40,	182	17.4	19,
177alhr.	69	75,	180	32	35,	183	14.2	16,
178	53.5	59,	181	24	26,	184b.	8.3	9,

Day 184. Drained and refilled with 250 ml. of 100 ppm. 2-CP.

Table 70. continued.

A.	B.	D.	A.	B.	D.	A.	B.	D.
184ba.	85.5	94,	187	32.2	35,	191	12.5	14,
184alhr.	68	74,	188	28	31,	192b.	8.4	9,
185	52.8	58,	189	20	22,			
186	/	/,	190	16.3	18,			
Day 192. Drained and refilled with 250 ml. of 100 ppm. 2-CP.								
192ba.	89	97,	194	36.3	40,	197b.	4	4,
192alhr.	74.5	81,	195	23.6	26,			
193	49.7	54,	196	12	13,			
Day 197. Drained and refilled with 250 ml. of 100 ppm. 2-CP.								
197ba.	92	101,	198	44	48,	200	12.1	13,
197alhr.	76	83,	199	35	38,	201b.	2.5	3,
Day 201. Drained and refilled with 250 ml. of 100 ppm. 2-CP.								
201ba.	85	93,	202	36.6	40,	204	2.9	3,
201alhr.	64	70,	203	14.3	16,			

Table 71. Direct perfusion of 2,4-DCP, followed by 4-CP.

Key to columns in table:
A. Day of perfusion.
B. Colourimeter reading (E.E.L. instrument,) in divisions.
C. Indicated 2,4-DCP concentration in the perfusate (ppm.).
D. Indicated 4-CP concentration in the perfusate (ppm.).

Perfusion started on 5/2/51 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried June 1951,) and 250 ml. of 50 ppm. 2,4-DCP solution. Solution sampled before adding to the perfuser and after 1 hr. perfusion.

A.	B.	C.	A.	B.	C.	A.	B.	C.
0ba.	25	50,	9	12.5	25,	19	6.5	13,
0alhr.	21	42,	10	11	22,	20	5.5	11,
1	20.6	41,	11	10.7	21,	21	8.2	16,
2	19.2	38,	12	8.1	16,	22	4.7	9,
3	19	38,	13	10.2	20,	23	6.5	13,
4	17	34,	14	8	16,	24	6.5	13,
5	16	32,	15	6.9	14,	25	5.3	10,
6	16	32,	16	7.2	14,	26b.	6.5	13,
7	15.5	31,	17	8	16,			
8	14.2	28,	18	7	14,			
Day 26. Drained and refilled with 250 ml. of 50 ppm. 2,4-DCP.								
26ba.	26.2	52,	27	16.8	34,	29b.	4.7	9,
26alhr.	17.9	36,	28	11.5	23,			
Day 29. Drained and refilled with 250 ml. of 60 ppm. 2,4-DCP.								
29ba.	29.8	60,	30	4.6	9,			
29alhr.	17.4	35,	31b.	1.7	3,			
Day 31. Drained and refilled with 250 ml. of 70 ppm. 2,4-DCP.								
31ba.	38.4	77,	31alhr.	23.8	48,	32b.	10	20,
Day 32. Drained and refilled with 250 ml. of 100 ppm. 2,4-DCP.								
32ba.	46.7	93,	33	16	32,			
32alhr.	30.3	61,	34b	4	8,			
Day 34. Drained and refilled with 250 ml. of 125 ppm. 2,4-DCP.								
34ba.	62	124,	36	28.4	57,	39b.	10	20,
34alhr.	41	82,	37	22.2	44,			
35	31.4	63,	38	15.3	31,			
Day 39. Drained and refilled with 250 ml. of 150 ppm. 2,4-DCP.								
39ba.	81	162,	42	38	76,	46	/	/,
39alhr.	59	118,	43	34.5	69,	47	11.7	23,
40	49	98,	44	27.6	55,	48	9.3	19,
41	40.2	80,	45	26	52,	49b.	3.4	7,
Day 49. Drained and refilled with 250 ml. of 100 ppm. 4-CP.								

Table 71. continued.

A.	B.	D.	A.	B.	D.	A.	B.	D.
49ba.	75.5	100,	55	31.5	42,	62	17	23,
49alhr.	49	65,	56	32	43,	63	14.1	19,
50	44	59,	57	33.1	44,	64	13	17,
51	40	53,	58	26.4	35,	65	/	/,
52	40.5	54,	59	29.3	39,			
53	36	48,	60	23.8	32,	82	4.1	5,
54	35	47,	61	24.8	33,	83b.	4	5,

Day 83. Drained and refilled with 250 ml. of 100 ppm. 4-CP.

83ba.	73	97,	85	39.6	53,	88	14.2	19,
83alhr.	50	67,	86	33.1	44,	89b.	3.3	4,
84	44.3	59,	87	25	33,			

Day 89. Drained and refilled with 250 ml. of 100 ppm. 4-CP.

89ba.	76	101,	91	27.8	37,
89alhr.	57	76,	92	16.2	22,
90	37	49,	93b.	6	8,

Day 93. Drained and refilled with 250 ml. of 100 ppm. 4-CP.

93ba.	74	98,	94	39.2	52,	96	20.1	27,
93alhr.	56	75,	95	29.7	40,	97b.	11.7	16,

Day 97. Drained and refilled with 250 ml. of 100 ppm. 2,4-DCP.

A.	B.	C.	A.	B.	C.	A.	B.	C.
97ba.	57	114,	98	29.8	60,	100b.	9	18,
97alhr.	41	82,	99	18.2	36,			

Day 100. Drained and refilled with 250 ml. of 100 ppm. 2,4-DCP.

100ba.	56.3	113,	101	21.8	44,	103	5	10,
100alhr.	40.5	81,	102	13.7	27,			

Table 71a. Direct perfusion of 2,4-DCP, followed by 4-CP.

Key to columns in table: as in Table 71, above.

Perfusion started on 19/12/51 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried June 1951,) and 250 ml. of 50 ppm. 2,4-DCP solution. Solution sampled before adding to the perfuser and after 1 hr. perfusion.

A.	B.	C.	A.	B.	C.	A.	B.	C.
0ba	24.8	50,	11	11	22,	15	8.4	17,
0alhr	21.7	43,	12	9.2	18,	16	8.9	18,
1	18	36,	13	9	18,	17	9.2	18,
2	14.5	29,	14	9.3	19,	18b	4.9	10,

Day 18. Perfuser drained and refilled with 250 ml. of 40 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

18ba	19.2	38,	19	8.8	18,	20b	5.4	11,
18alhr	11.8	24,						

Day 20. Perfuser drained and refilled with 250 ml. of 50 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

20ba	24.5	49,	21	11.2	22,	23b	0	0,
20alhr	15	30,	22	4.3	9,			

Day 23. Perfuser drained and refilled with 250 ml. of 60 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

23ba	34.3	69,	24	11.3	23,	25b	6.1	12,
23alhr	19	38,						

Day 25. Perfuser drained and refilled with 250 ml. of 70 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

25ba	35.6	71,	26	17.8	36,	28b	4.2	8,
25alhr	25.1	50,	27	8.1	16,			

Day 28. Perfuser drained and refilled with 250 ml. of 90 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

28ba	49	98,	29	21.1	42,	31	9	18,
28alhr	32.3	65,	30	13.6	27,	32b	1.2	2,

Day 32. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

32ba	53	106,	33	18	36,	34b	10.1	20,
32alhr	28	56,						

Day 34. Perfuser drained and refilled with 250 ml. of 125 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

34ba	66	132,	35	30	60,	36b	10	20,
34alhr	37	74,						

Table 71a. continued.

Day 36. Perfuser drained and refilled with 250 ml. of 175 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

A.	B.	C.	A.	B.	C.	A.	B.	C.
36ba	87	174,	37	35.5	71,	38b	14.1	28,
36alhr	52	104,						

Day 38. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

38ba	100.5	201,	39	55	110,	41	23.2	46,
38alhr	78	156,	40	38	76,	42b	6	12,

Day 42. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

42ba	83	166,	43	41.5	83,	45b	7	14,
42alhr	64	128,	44	26	52,			

Day 45. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

45ba	100	200,	47	38.2	76,	50b	5.2	10,
45alhr	64	128,	48	25	50,			
46	46.5	93,	49	16.6	33,			

Day 50. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

50ba	103	206,	52	43.5	87,	55	23.2	46,
50alhr	68	136,	53	37.2	74,	56	15.9	32,
51	52	104,	54	30.2	60,	57b	5.7	11,

Day 57. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

57ba	102	204,	60	43	86,	64	20	40,
57alhr	70	140,	61	38.3	77,	65b	12.6	25,
58	53	106,	62	32.4	65,			
59	44	88,	63	22.8	46,			

Day 65. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

65ba	100	200,	69	45	90,	74	22	44,
65alhr	64.5	129,	70	35.3	71,	75	19.1	38,
66	57	114,	71	38.4	77,	76	12.3	25,
67	51	102,	72	34.5	69,	77b	5	10,
68	49	98,	73	25.3	51,			

Day 77. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

77ba	94	188,	78	55	110,	80	47	94,
77alhr	65	130,	79	51	102,	81	38	76,

Table 71a. continued.

A.	B.	C.	A.	B.	C.	A.	B.	C.
82	35	70,	84	26	52,	86	17.7	35,
83	28	56,	85	22	44,	87b	14.8	30,

Day 87. Perfuser drained and refilled with 250 ml. of 150 ppm. 4-CP solution. Sampled before adding and after 1 hr. perfusion.

87ba	110	146,	104	48	64,	139	28.4	38,
87alhr	86	114,	105	47.2	63,	140	29.2	39,
88	76	101,	106	42.6	57,	141	27.1	36,
89	62.5	83,	107	44	59,	142	26.7	36,
90	63	84,	108	40.3	54,	143	26.4	35,
91	66	88,	109	44	59,	144	23.9	32,
92	60.6	81,	110	39.6	53,	145	23.2	31,
93	55	73,	111	37.5	50,	146	20.0	27,
94	/	/,	112	38.4	51,	147	20.4	27,
95	51.5	69,	130	29.1	39,	148	17.7	24,
96	53.3	71,	131	31	41,	149	14.2	19,
97	49	65,	132	33.1	44,	150	13.5	18,
98	48.5	65,	133	31.1	41,	151	10.2	14,
99	51	68,	134	30.2	40,	152	11.3	15,
100	50	67,	135	32.2	43,	153	10.6	14,
101	46	61,	136	28.4	38,	154	12.3	16,
102	42.5	57,	137	32	43,			
103	41	55,	138	29	39,			

Table 72. Direct perfusion of 2,4-DCP, followed by 2,4-D and later by 2,4-D / 2,4-DCP mixture.

Key to columns in table:
A. Day of perfusion.
B. Colourimeter reading (Unicam instrument,) in divisions.
C. Indicated 2,4-DCP concentration in the perfusate (ppm.).
D. Mean control root length (mm.).
E. Longest roots at nominal dilution concentration of 0.01 ppm. (relative to the 2,4-D component,) as % mean control.
F. Longest roots at nominal dilution concentration of 0.1 ppm. (relative to the 2,4-D component,) as % mean control.
G. Longest roots at nominal dilution concentration of 1.0 ppm. (relative to the 2,4-D component,) as % mean control.
H. Indicated 2,4-D concentration, or total activity as 2,4-D, in the perfusate (ppm.).

Perfusion started on 27/2/53 with 50 gm. of soil (2.to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 100 ppm. 2,4-DCP solution (common with solutions in Tables 72a and 72d,). Solution sampled before adding to the perfuser and after 1 hr.

A.	B.	C.	A.	B.	C.	A.	B.	C.
0ba.	26	88,	5	56.1	38,	11	84.2	12,
0alhr.	35	68,	6	59.6	34,	12	85.8	10,
1	42	57,	7	67	26,	13	88.4	8,
2	44.5	53,	8	75.3	19,	14	90	7,
3	49.5	46,	9	78.2	16,	15	90.6	6,
4	52	43,	10	81.9	13,	16b.	90.8	6,
Drained and refilled with 250 ml. of 60 ppm. 2,4-DCP. Day 16.								
16ba.	38	63,	17	56.1	38,	19b.	85.4	11,
16alhr.	51.1	44,	18	66.4	27,			
Day 19. Drained and refilled with 250 ml. of 100 ppm. 2,4-DCP.								
19ba.	20.3	104,	20	50.6	45,	22b.	94.3	4,
19alhr.	32.3	74,	21	88.5	8,			
Day 22. Drained and refilled with 250 ml; of 100 ppm. 2,4-DCP.								
22ba.	20.6	103,	23	51.0	44,	25b.	95.0	4,
22alhr.	31.3	76,	24	76.9	17,			
Day 25. Drained and refilled with 250 ml. of 100 ppm. 2,4-DCP.								
25ba.	19.6	106,	26	48.2	47,	28	87.1	9,
25alhr.	32.7	73,	27	67	26,	29b.	95.5	4,
Day 29. Drained and refilled with 250 ml. of 100 ppm. 2,4-DCP.								
29ba.	21.7	100,	31	64.7	29,	34	96.3	3,
29alhr.	36	67,	32	80.8	14,	35b.	100	0,
30	47	50,	33	94	4,			
Day 35. Drained and refilled with 250 ml. of 100 ppm. 2,4-DCP.								

Table 72. continued.

A.	B.	C.	A.	B.	C.	A.	B.	C.
35ba.	23.5	96,	37	61.7	32,	40b.	97.0	2,
35alhr.	36.5	66,	38	76.4	18,			
36	49.6	46,	39	95.5	3,			

Day 40. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re- started, sampled after 1 hr.

A.	D.	E. E. E. E.	F. F. F. F.	G. G.	H.
40	83.4	47, 48, 58, 58,	13, 13, 17, 17,	2.5,2.5,	140,
45	77.9	65, 71, 76,107,	21, 21, 24, 26,	5,6.5,	100,
50	74.6	100.102,103,108,	72, 76, 80, 83,	19, 23,	12,
55	76.7	94, 94,100,109,	98,102,103,106,	34, 39,	5,
60b	78.5	102,102,107,116,	97,108,109,120,	94,120,	<1

Day 60. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

60a.	78.5	80, 80, 85, 94,	19, 19, 22, 23,	6.5,7.5,	100,
63	75.5	84, 87, 90,104,	24, 24, 24, 25,	5.5,8.5,	85,
66b	81.4	94, 94, 95, 98,	90, 92, 95,103,	109,110,	<1,

Day 69. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

69a	84.3	70, 71, 80, 80,	17, 20, 21, 21,	3.5,3.5,	115,
72	88.6	98,101,107,111,	105,106,111,111,	103,104,	<1,

Day 75. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 100 ppm. 2,4-DCP. Sampled for phenols before adding to the perfuser and for phenols and 2,4-D after 1 hr. perfusion.

75	75.1	70, 74, 76, 77,	17, 18, 21, 25,	1.5,2.5,	120,
78	69.4	106,112,118,118,	29, 30, 34, 36,	8.5, 12,	50,
81	87.6	93, 95, 96,104,	96, 99,103,111,	96,100,	<1,

Day 84. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 200 ppm. 2,4-DCP. Sampled for phenols before adding and for phenols and 2,4-D after 1 hr.

84a	88.3	77, 80, 91, 98,	17, 18, 23, 26,	2.5,2.5,	110,
87	89.8	67, 77, 85, 95,	16, 19, 19, 20,	4.5,4.5,	120,
90	95.0	70, 73, 76, 90,	16, 18, 18, 19,	3,5.5,	130,
93	96.9	91, 96,103,106,	92, 93, 94,107,	86, 93,	<1,

A.	B.	C.	A.	B.	C.	A.	B.	C.
75ba.	23.3	95,	75alhr.	31.5	75,	76	51.2	44,
77	69.5	24,	78	93.6	4,	79	96.7	2,
80	96.8	2,	81	97.3	2,	82	97.1	2,
83	96.8	2,	84b.	98.8	1,	84ba.	6.7	177,
84alhr.	8.1	164,	85	20.4	104,	86	23.9	94,
87	27	85,	88	30	79,	89	67.8	26,
90	92.2	5,	91	93.8	4,	92	95.4	3,
93	94.6	4,	94	94.9	4,			

Table 72a. Direct perfusion of 2,4-DCP, followed by 2,4-D and later by 2,4-D / 2,4-DCP mixture.

Key to columns in table: as in Table 72, above.

Details of perfusion set-up: as for Table 72. 2,4-DCP solution common with that in Tables 72 and 72d. Solution sampled before adding to the perfuser and after 1 hr. perfusion.

A.	B.	C.	A.	B.	C.	A.	B.	C.
0ba	26	88,	5	51.2	44,	11	79.1	15,
0alhr	34.5	69,	6	53.6	41,	12	79.8	14,
1	40.5	59,	7	58	36,	13	82.3	13,
2	43.5	54,	8	62.1	31,	14	87	9,
3	46.5	50,	9	67.4	26,	15	87.7	8,
4	47.4	48,	10	73.4	20,	16b	90.2	7,

Day 16. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

16ba	38	63,	17	57.8	36,	19b	92.1	6,
16alhr	52.8	42,	18	71.4	22,			

Day 19. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

19ba	20.3	104,	20	43.8	54,	22b	77	17,
19alhr	32.4	73,	21	62.2	31,			

Day 22. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

22ba	20.6	103,	23	54.1	40,	25b	84.4	11,
22alhr	30.5	78,	24	71.8	22,			

Day 25. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

25ba	19.6	106,	26	44.6	53,	28	72	22,
25alhr	28.3	82,	27	59.4	34,	29b	74.4	19,

Day 29. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

29ba	21.7	100,	31	59.0	34,	34	84.8	11,
29alhr	34	70,	32	65.3	28,	35b	96.8	2,
30	46.3	51,	33	76.3	18,			

Day 35. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

35ba	23.5	94,	37	55.6	38,	40b	74.8	19,
35alhr	32.3	74,	38	61.4	32,			
36	48.4	47,	39	72.9	21,			

Table 72a. continued.

Day 40. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

A.	D.	E. E. E. E.	F. F. F. F.	G. G.	H.
40a	83.4	56, 57, 63, 67,	17, 17, 18, 23,	2.5,3.5,	130
45	77.9	53, 53, 62, 68,	14, 15, 20, 22,	2.5, 4,	130
50	74.6	65, 69, 73, 77,	21, 23, 24, 24,	4,5.5,	95
55	76.7	75, 79, 79, 83,	35, 36, 40, 42,	6.5, 8,	50
60b	78.5	95,104,107,113,	101,102,113,121,	101,111,	<1

Day 60. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

60a	78.5	76, 77, 88, 89,	15, 16, 17, 18,	4, 5,	100
63	75.5	96, 99,102,104,	104,106,107,116,	80, 80,	1
66	81.4	100,101,103,106,	95, 97, 97,101,	93, 97,	<1

Day 69. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

69a	84.3	86, 93, 96, 98,	19, 20, 24, 25,	3.5,4.5,	100
72	88.6	99,104,106,109,	98,103,103,106,	94, 95,	<1

Day 75. Perfuser drained and refilled with 250 ml. of solution containing 100 ppm. 2,4-D and 200 ppm. 2,4-DCP. Solution sampled for 2,4-DCP before adding to the perfuser and for 2,4-D and 2,4-DCP after 1 hr. perfusion.

75a	75.1	78, 82,100,104,	14, 17, 23, 25,	1.5,2.5,	120
78	69.4	80, 83, 86, 87,	16, 17, 18, 22,	3,4.5,	120
81	87.6	73, 77, 81, 89,	15, 17, 17, 17,	2.5,4.5,	120
84	88.3	67, 69, 76, 79,	16, 17, 19, 20,	2.5,3.5,	130
87	89.8	68, 69, 76, 86,	16, 17, 19, 19,	2,3.5,	130
90	95.0	69, 73, 76, 79,	13, 15, 16, 21,	3, 3,	130
93	96.9	75, 75, 88, 90,	21, 22, 25, 28,	3, 3,	105
96	100.4	64, 70, 78, 89,	12, 12, 13, 16,	2, 2,	130
99	95.7	78, 79, 80, 93,	15, 17, 18, 21,	3, 4,	115
102	100.5	86, 90, 93,102,	20, 20, 22, 24,	3, 3,	100
108	91.7	103,107,117,121,	94,101,102,102,	101,105,	<1
111b	93.7	89, 91, 92,100,	86, 86, 86, 91,	87, 88,	<1

Day 111. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

111a	93.7	68, 69, 78, 88,	17, 17, 18, 21,	4.5,5.5,	110
114	104.1	89, 89, 90, 94,	98,101,103,106,	79, 99,	<1

Phenol concentrations in the perfusate during the period of perfusion with 2,4-D / 2,4-DCP mixture.

A.	B.	C.	A.	B.	C.	A.	B.	C.
75ba	6.4	291,	76	18.9	109,	78	20.1	104,
75alhr	11.9	139,	77	19	108,	79	20.7	103,

Table 72a. continued.

A.	B.	C.	A.	B.	C.	A.	B.	C.
80	23.1	96,	85	28	83,	90	36.6	66,
81	24.7	91,	86	29	81,	91	39	61,
82	25.5	89,	87	30.8	77,	92	46.6	50,
83	26.4	87,	88	33.1	72,	93	59.3	34,
84	27.1	85,	89	34.1	70,	94	83.9	12,

Table 72b. Direct perfusion of 2,4-DCP, followed by 2,4-D.

Key to columns in table:
A. Day of perfusion.
B. Colourimeter reading (E.E.L. Instrument.) in divisions.
C. Indicated 2,4-DCP concentration in the perfusate (ppm.).
D. Mean control root length (mm.).
E. Longest roots at nominal dilution concentration of 0.01 ppm. as percentage of mean control.
F. Longest roots at nominal concentration of 0.1 ppm. as % m.c.
G. Longest roots at nominal concentration of 1.0 ppm. as % m.c.
H. Indicated 2,4-D concentration in the perfusate (ppm.).

Perfusion started on 25/5/51 with 75 gm. of soil (1 to 4 mm., South Church Lane, Bishop Auckland, allotment soil, dried January 1951) and 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding to perfuser and after 1 hr. Solution common with that of Table 72c, but separate initial samples were taken.

A.	B.	C.	A.	B.	C.	A.	B.	C.
0ba	97	194,	7	39.8	80,	15	34.5	69,
0alhr	54.5	109,	8	42.4	85,	16	31.2	62,
1	52	104,	9	40.7	81,	17	33	66,
2	46	92,	10	37.4	75,	18	31.2	62,
3	40.4	81,	11	36.6	73,	19	29.5	59,
4	45.2	90,	12	37	74,	20	27	54,
5	43	86,	13	36.8	74,	38	4.3	8,
6	43	86,	14	33.6	67,			

Day 40. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

40ba	106	212,	43	45.6	91,	47	15.2	30,
40alhr	70	140,	44	43	86,	48b	5.4	11,
41	55.5	111,	45	39	78,			
42	51.2	102,	46	28.2	56,			

Table 72b. continued.

A.	B.	C.	A.	B.	C.	A.	B.	C.
Day 48. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.								
48ba	106	212,	50	48.5	97,	53	20.1	40,
48alhr	68.5	137,	51	40	80,	54b	6	12,
49	54.5	109,	52	33.2	66,			
Day 54. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.								
54ba	97	194,	55	49	94,	57b	14.2	28,
54alhr	67	134,	56	27	54,			
Day 57. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.								
57ba	113	226,	59	54	108,	62	23.2	46,
57alhr	79	158,	60	45.4	91,	63b	12	24,
58	59.4	119,	61	37	74,			
Day 63. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.								
63ba	118	236,	65	31.5	63,	68b	4.5	9,
63alhr	78	156,	66	20.7	41,			
64	48	96,	67	14.5	29,			
Day 68. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.								
68ba	107	214,	70	30.4	61,	73b	7.2	14,
68alhr	75.5	151,	71	20.4	41,			
69	43	86,	72	14	28,			
Day 73. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.								
73ba	103	206,	75	36.8	74,	78b	16	32,
73alhr	83	166,	76	30.2	60,			
74	54.2	108,	77	21.2	42,			
Day 78. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.								
78ba	75	150,	79	39	78,	81b	21.5	43,
78alhr	50	100,	80	33	66,			
Day 81. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.								
81ba	108	216,	83	63	126,	86	34.2	68,
81alhr	85	170,	84	54	108,	87	23.1	46,
82	72	144,	85	46	92,	88b	20	40,

Table 72b. continued.

Day 88. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

A.	B.	C.	A.	B.	C.	A.	B.	C.
88ba	107	214,	90	52	104,	93	21.2	42,
88alhr	84	168,	91	38	76,	94b	17.2	34,
89	57.5	115,	92	28	56,			

Day 94. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

94ba	107	214,	96	55.5	111,	99	28.5	57,
94alhr	72	144,	97	44.5	89,	100	24.5	49,
95	59	118,	98	38	76,	101b	17	34,

Day 101. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

101ba	96	192,	103	46	92,	106	22.5	45,
101alhr	77	154,	104	33.5	67,	107	14.5	29,
102	52.5	105,	105	26	52,	108b	11.8	24,

Day 108. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

108ba	111	222,	111	50.5	101,	115	21.6	43,
108alhr	90	180,	112	39	78,	116	18.5	37,
109	70	140,	113	32.6	65,	117b	14	28,
110	60	120,	114	24.3	49,			

Day 117. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

117ba	93	186,	119	41.5	83,	122	29	58,
117alhr	70	140,	120	42.5	85,	123b	25.1	50,
118	54	108,	121	35	70,			

Day 123. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

123ba	96	192,	126	46.5	93,	130	23	46,
123alhr	85	170,	127	40.5	81,	131	20.2	40,
124	61	122,	128	35.3	71,	132b	17.3	35,
125	50	100,	129	29	58,			

Day 132. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

A.	D.	E.	E.	E.	E.	F.	F.	F.	F.	G.	G.	H.
132a	69.1	67,	70,	74,	84,	19,	20,	26,	45,	4.5,	4.5,	120
135	51.6	62,	75,	79,	89,	19,	19,	23,	27,	2,	3,	120
137	72.3	54,	55,	55,	90,	21,	21,	24,	32,	4,	7,	110
139	59.8	67,	72,	72,	80,	28,	32,	33,	35,	3.5,	5,	85
141	59.3	83,	89,	93,	96,	22,	24,	30,	44,	5,	6.5,	65

Table 72b. continued.

Day 142. Perfuser drained in error and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Sampled after 1 hr. perfusion.

A.	D.	E.	E.	E.	E.	F.	F.	F.	F.	G.	G.	H.
142a	67.7	78,	81,	83,	100,	22,	22,	35,	38,	3,	6,	1,000
148	43.4	83,	95,	99,	127,	35,	35,	37,	39,	4.5,	4.5,	600
150	44.2	88,	95,	97,	102,	34,	36,	36,	39,	7,	16,	500
152	43.2	104.	107,	123,	130,	32,	35,	37,	49,	4.5,	12,	550
154	43.1	74,	79,	79,	100,	30,	33,	33,	37,	7,	7,	600
156	49.8	74,	78,	90,	104,	26,	26,	30,	32,	4,	6,	700
158	64.0	59,	63,	67,	85,	20,	22,	25,	36,	4.5,	6.5,	1,000
160	60.7	56,	58,	64,	64,	21,	21,	23,	23,	6.5,	8,	1,000
162	66.8	88,	102,	106,	115,	22,	24,	31,	42,	4.5,	6,	650
164	63.8	61,	61,	64,	94,	31,	31,	33,	38,	4.5,	6.5,	650
166	71.5	74,	102,	108,	111,	29,	31,	34,	35,	4,	8.5,	650
168	70.7	55,	65,	66,	78,	23,	24,	33,	38,	4,	7,	700
170	62.8	104,	105,	107,	118,	24,	28,	28,	37,	3,	3,	700
172	75.2	83,	85,	95,	111,	21,	23,	27,	32,	2.5,	4,	850
174	79.1	54,	73,	77,	93,	20,	23,	23,	33,	2.5,	4,	900
176	75.2	61,	63,	64,	92,	23,	24,	32,	47,	3,	4,	900
178	81.6	54,	71,	80,	106,	15,	15,	23,	25,	1,	2.5,	900
180	69.5	59,	59,	61,	99,	13,	14,	16,	19,	3,	3,	1,200
182	59.4	62,	74,	79,	98,	25,	27,	30,	37,	6.5,	10,	700
184	55.9	83,	83,	92,	111,	27,	32,	34,	36,	5.5,	5.5,	600
186	68.3	59,	67,	72,	101,	21,	21,	21,	37,	3,	4.5,	1,000
188	66.0	74,	86,	87,	87,	27,	30,	30,	41,	4.5,	6,	750
190	78.0	77,	85,	85,	89,	17,	17,	18,	19,	2.5,	4,	900
193	67.6	73,	73,	73,	92,	18,	19,	24,	25,	4.5,	6,	1,150
196	76.3	63,	76,	80,	98,	20,	23,	23,	26,	3.5,	5,	1,000
199	75.4	48,	57,	60,	80,	23,	24,	24,	33,	4,	5.5,	900
202	71.4	72,	74,	83,	90,	28,	29,	34,	48,	4,	5.5,	700
206	48.8	90,	94,	97,	123,	35,	37,	43,	47,	8,	15,	450

Table 72c. Direct perfusion of 2,4-DCP, followed by 2,4-D.

Key to columns in table: as in Table 72b, above,

Details of perfusion set-up: as in Table 72b, above. A common solution was divided between the perfusers of Tables 72b and 72c but individual samples were taken both before adding to the perfuser and after 1 hr. perfusion.

A.	B.	C.	A.	B.	C.	A.	B.	C.
Oba	98	196,	1	48.8	98,	3	43	86,
Oalhr	54.4	109,	2	44	88,	4	42.3	85,

Table 72c. continued.

A.	B.	C.	A.	B.	C.	A.	B.	C.
5	43	86,	11	38.1	76,	17	29.0	58,
6	43	86,	12	36.5	73,	18	27.9	56,
7	39.2	78,	13	35.0	70,	19	23.1	46,
8	40.7	81,	14	33	66,	20	19.7	39,
9	39.6	79,	15	31.5	63,	38	6.4	13,
10	37.4	75,	16	28.0	56,			

Day 40. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

40ba	106	212,	43	45.6	91,	47	18	36,
40alhr	66	132,	44	41	82,	48b	6.8	14,
41	53.5	107,	45	34	68,			
42	48	96,	46	27.4	55,			

Day 48. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

48ba	106	212,	51	53	106,	55	27	54,
48alhr	76	152,	52	48.1	96,	56	9.1	18,
49	60	120,	53	40.6	81,	57b	4.5	9,
50	55	110,	54	35.1	70,			

Day 57. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

57ba	113	226,	59	48.5	97,	62	24.6	49,
57alhr	70	140,	60	42.0	84,	63b	19.5	39,
58	55.4	111,	61	32.0	64,			

Day 63. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

63ba	118	236,	65	50.5	101,	68b	22.5	45,
63alhr	78	156,	66	47	94,			
64	63	126,	67	36.7	73,			

Day 68. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

68ba	107	214,	70	53	106,	73b	20.6	41,
68alhr	74.5	149,	71	40	80,			
69	60	120,	72	33.6	67,			

Day 73. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

73ba	103	206,	76	48.6	97,	80	18.9	38,
73alhr	85	170,	77	40	80,	81b	13.2	26,
74	67	134,	78	35.6	71,			
75	54.5	109,	79	25	50,			

Day 81. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

Table 72c. continued.

A.	B.	C.	A.	B.	C.	A.	B.	C.
81ba	108	216,	83	55	110,	86	29	58,
81alhr	78	156,	84	44	88,	87	21	42,
82	66	132,	85	35.5	71,	88b	18	36,

Day 88. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

88ba	107	214,	91	39	78,	95	24	48,
88alhr	71	142,	92	39.5	79,	96b	23.5	47,
89	60.5	121,	93	34	68,			
90	51.5	103,	94,	30	60,			

Day 96. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

96ba	101	202,	98	45	90,	101b	29.1	58,
96alhr	72	144,	99	41.5	83,			
97	59	118,	100	36.5	73,			

Day 101. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

101ba	96	192,	103	51	102,	106	35	70,
101alhr	76	152,	104	46	92,	107	29	58,
102	64	128,	105	40.5	81,	108b	24	48,

Day 108. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

108ba	111	222,	112	48	96,	117	23.5	47,
108alhr	95	190,	113	40	80,	118	23.3	47,
109	79	158,	114	34	68,	119b	20.3	41,
110	66	132,	115	33.5	67,			
111	57	114,	116	27.7	55,			

Day 119. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

119ba	115	230,	123	56.5	113,	128	31.7	63,
119alhr	79	158,	124	45.5	91,	129	28.4	57,
120	66.5	133,	125	40.5	81,	130	23	46,
121	67	134,	126	32.3	65,	131	21.7	43,
122	61.5	123,	127	32	64,	132b	18.1	36,

Day 132. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

A.	D.	E.	E.	E.	E.	F.	F.	F.	F.	G.	G.	H.
132a	69.1	70,	70,	77,	81,	20,	22,	29,	35,	4.5,	8.5,	80
135	51.6	64,	64,	82,	113,	19,	21,	25,	25,	3,	5,	90
137	72.3	62,	71,	78,	89,	19,	24,	24,	25,	3,	4,	95
139	59.8	48,	50,	58,	75,	28,	32,	33,	35,	5,	6.5,	65
141	59.3	74,	78,	89,	91,	24,	25,	27,	47,	3.5,	5,	85

Table 72c. continued.

A.	D.	E.	E.	E.	E.	F.	F.	F.	F.	G.	G.	H.
143	67.7	71,	78,	84,	84,	24,	27,	31,	32,	4.5,	6,	75
145	69.9	66,	84,	89,	93,	20,	29,	29,	30,	4,	6,	70
147	36.5	85,	93,	96,	96,	44,	44,	52,	52,	8,	8,	40
149	60.9	90,	94,	105,	114,	58,	61,	71,	74,	15,	18,	15
151	51.7	72,	72,	76,	77,	70,	70,	85,	106,	95,	110,	<1

Day 153. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

153a	53.3	81,	96,	98,	100,	23,	24,	26,	26,	3,	3,	900
157	47.7	67,	72,	80,	84,	23,	26,	26,	30,	4,	4,	800
159	72.3	64,	83,	87,	91,	22,	25,	25,	33,	4,	5.5,	850
161	66.1	73,	74,	83,	83,	23,	26,	28,	38,	4.5,	7.5,	850
162	63.8	77,	82,	85,	116,	22,	24,	25,	41,	3,	6.5,	900
165	66.4	71,	80,	80,	83,	23,	24,	27,	27,	3,	3,	900
167	64.4	78,	87,	104,	126,	20,	22,	22,	25,	4.5,	6,	1,000
169	68.3	51,	73,	81,	92,	21,	22,	29,	32,	3,	4.5,	900
171	67.0	67,	70,	75,	97,	16,	19,	21,	21,	3,	4.5,	1,050
173	74.7	80,	83,	88,	97,	19,	20,	20,	23,	3,	3,	1,100
175	75.2	68,	79,	79,	87,	17,	21,	21,	24,	4,	4,	1,050
177	72.9	68,	73,	77,	80,	22,	23,	26,	32,	1.5,	4,	950
179	78.6	51,	54,	70,	74,	18,	19,	20,	22,	2.5,	4,	1,100
181	62.2	58,	68,	76,	79,	26,	27,	34,	35,	3,	3,	900
183	64.1	39,	42,	51,	58,	17,	17,	19,	19,	3,	4.5,	1,200
185	52.7	78,	80,	85,	97,	17,	19,	23,	29,	4,	5.5,	1,000
187	59.7	72,	88,	92,	93,	22,	23,	25,	30,	3.5,	3.5,	900
189	61.6	88,	88,	99,	117,	16,	16,	20,	21,	1.5,	3.5,	1,150
191	58.6	68,	74,	82,	91,	21,	21,	22,	31,	3.5,	5,	1,000

Table 72d. Direct perfusion of 2,4-DCP, followed by 2,4-D.

Key to columns in table: as in Table 72, above.

Perfusion started on 27/2/53 with 50 gm. of soil (2 to 4 mm., Sussex Lodge soil, dried January 1953,) and 250 ml. of 100 ppm. 2,4-DCP solution (common with solutions in Tables 72 and 72a,). Solution sampled before adding to the perfuser and after 1 hr.

A.	B.	C.	A.	B.	C.	A.	B.	C.
0ba.	26	88,	5	52.7	42,	11	83	12,
0alhr.	34	70,	6	54.6	40,	12	85.3	10,
1	40.5	59,	7	60.9	32,	13	85.4	10,
2	44	54,	8	67.4	26,	14	86.5	9,
3	47.5	49,	9	72.5	21,	15	89.8	7,
4	51.3	44,	10	78.5	16,	16b.	91.0	6,

Day 16. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr. perfusion.

16ba.	38	63,	17	56.9	37,	19b.	84.1	11,
16alhr.	52.4	42,	18	64	29,			

Day 19. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

19ba.	20.3	104,	20	46.6	50,	22b.	78.7	21,
19alhr.	31.1	76,	21	59.7	34,			

Day 22. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

22ba.	20.6	103,	23	42.7	56,	25b.	67.7	26,
22alhr.	30.4	78,	24	54.8	39,			

Day 25. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

25ba.	19.6	106,	26	40.1	60,	28	67.7	26,
25alhr.	30.4	78,	27	49.9	45,	29b.	83.4	12,

Day 29. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

29ba.	21.7	100,	31	60.3	33,	34	92.3	5,
29alhr.	36.3	66,	32	72.7	21,	35b.	98.7	1,
30	45.9	51,	33	87.6	9,			

Day 35. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

35ba.	23.5	95,	37	50.1	45,	40	76.1	18,

Table 72d. continued.

A.	B.	C.	A.	B.	C.	A.	B.	C.
35alhr.	34.6	69,	38	58.0	35,			
36	38.4	62,	39	66.4	27,			

Day 40. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

A.	D.	E.	E.	E.	E.	F.	F.	F.	F.	G.	G.	H.
40a	83.4	38,	43,	44,	47,	17,	17,	19,	19,	1,	2.5,	130
45	77.9	54,	58,	63,	70,	16,	17,	18,	26,	4,	4,	120
50	74.6	65,	68,	75,	79,	17,	18,	19,	20,	5.5,	8,	130
55	76.7	98,	104,	117,	121,	60,	61,	70,	78,	17,	17,	15
60	78.5	109,	113,	113,	116,	103,	104,	118,	134,	94,	117,	<1

Day 60. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

60a	78.5	76,	81,	86,	94,	18,	18,	19,	23,	2.5,	4,	120
63	75.5	91,	91,	99,	106,	101,	101,	106,	111,	90,	103,	<1
66	81.4	84,	92,	94,	100,	95,	98,	100,	108,	79,	90,	<1

Day 69. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

69a	84.3	78,	83,	84,	86,	18,	18,	18,	19,	2.5,	2.5,	115
72	88.6	90,	91,	92,	99,	92,	95,	95,	95,	87,	93,	<1

Day 75. Perfuser drained and refilled with 250 ml. of 100 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

A.	B.	C.	A.	B.	C.	A.	B.	C.
75ba.	20.9	102,	77	64.6	29,	80	97.0	2,
75alhr.	33.4	71,	78	96	3,	81b.	97.7	2,
76	52.3	42,	79	97.5	2,			

Day 81. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Sampled before adding and after 1 hr.

81ba.	7.5	188,	85	41.9	57,	90	91.9	5,
81alhr.	11.9	139,	86	53.9	40,	91	93.7	4,
82	21.2	101,	87	82.9	12,	92	94.2	4,
83	22.2	98,	88	87.6	9,	93	94.0	4,
84	32.7	73,	89	87.7	9,	94	93.8	4,

Table 72e. Direct perfusion of 2,4-DCP, followed by 2,4-D.

Key to columns in table: as in Table 72, above.

Perfusion started on 16/2/51 with 100 gm. of soil (1 to 4 mm., Sussex Lodge soil, dried September 1950,) and 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding to the perfuser and after 1 hr. perfusion.

A.	B.	C.	A.	B.	C.	A.	B.	C.
0ba	96.5	193,	4	30	60,	9	21.4	43,
0alhr	48	96,	5	29.2	58,	10	21	42,
1	35.7	71,	6	27.2	54,	11	18.2	36,
2	32.9	66,	7	25.0	50,	12	12.9	26,
3	32.9	66,	8	24.0	48,	13b	10	20,

Day 13. 25 ml. of 2,000 ppm. 2,4-DCP solution added to the perfuser without draining, making the perfusate approximately 250 ml. of 200 ppm. 2,4-DCP. Sampled after 1/2 hr. perfusion.

13a	70	140,	18	40.9	82,	23	34.8	70,
14	49.4	99,	19	40.9	82,	24	34	68,
15	48.1	96,	20	39.0	78,	25	34	68,
16	44	88,	21	39.0	78,	26b	34.4	69,
17	42.6	85,	22	37.2	74,			

Day 26. 25 ml. of 2,000 ppm. 2,4-DCP solution added to the perfuser without draining, making the perfusate approximately 250 ml. of 200 ppm. 2,4-DCP. Sampled after 1/4 hr. perfusion.

26a	102	204,	49	46.8	94,	60	39	78,
27	80	160,	50	41.5	83,	61	36.9	74,
28	79	158,	51	46	92,	62	35	70,
29	75.5	151,	52	44	88,	63	35.8	72,
30	71	142,	53	39.5	79,	64	36.6	73,
31	67	134,	54	43.5	87,	65	32.7	65,
32	64.1	128,	55	42	84,	66	28.8	58,
33	59	118,	56	43.5	87,	67	15	30,
46	43	86,	57	40	80,	68b	7.4	15,
47	46	92,	58	40	80,			
48	46	92,	59	37.1	74,			

Day 68. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Perfuser re-started, sampled after 1 hr.

68alhr	42.2	84,	72	30	60,	76	22.0	44,
69	40	80,	73	30	60,	77	4.9	10,
70	42	84,	74	26.4	53,	78b	6.6	13,
71	35.8	72,	75	20.8	42,			

Day 78. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Perfuser re-started, sampled after 1 hr.

78alhr	57	114,	81	32.1	64,	84	16.9	34,
79	39.4	69,	82	28.5	57,	85b	6.6	14,
80	36.7	73,	83	23.7	47,			

Table 72e. continued.

Day 85. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Sampled after 3 hrs. perfusion.

A.	B.	C.	A.	B.	C.	A.	B.	C.
85a3hr	48	96,	87	34.2	68,	89	15.9	32,
86	39	78,	88	27	54,	90b	9	18,

Day 90. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Sampled after 2 hr. perfusion.

90a2hr	52	104,	92	36	72,	94	11.6	23,
91	43	86,	93	22.9	46,	95b	9.2	18,

Day 95. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Sampled after 1 hr.

95a1hr.	55	110,	97	19.4	39,	98b	12.5	25,
96	33.4	67,						

Day 98. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

A.	D.	E. E. E. E.	F. F. F. F.	G. G.	H.
98a	34.1	97,102,117,138,	44, 47, 53, 56,	6, 6,	500
99	34.1	73, 79, 97,100,	47, 50, 50, 56,	9, 9,	350
100	42.4	83, 85, 85, 87,	43, 47, 47, 57,	7, 14,	350
101	40.2	102,104,110,114,	40, 40, 47, 52,	7.5, 10,	350
102	40.2	85, 87, 92, 97,	45, 50, 55, 60,	7.5,7.5,	300
103	42.8	63, 63, 75, 84,	33, 35, 44, 49,	7, 17,	500
104	40.0	80, 85, 95,105,	43, 43, 50, 53,	7.5, 13,	300
105	45.2	71, 73, 82, 84,	42, 42, 51, 51,	4.5, 11,	400
106	45.2	64, 71, 77, 80,	44, 47, 47, 51,	6.5, 9,	350
107	45.2	82, 84,100,117,	35, 38, 38, 60,	6.5, 13,	500
108	38.8	93,103,103,108,	39, 39, 41, 49,	5, 10,	500
109	38.8	90, 93, 93, 95,	44, 46, 49, 52,	5, 5,	500
110	38.8	44, 46, 52, 95,	39, 39, 41, 44,	7.5, 10,	500
111	38.8	52, 52, 57, 62,	34, 36, 54, 64,	7.5, 13,	350
112	40.7	69, 71, 71, 71,	39, 39, 47, 52,	12, 12,	450
113	40.7	86, 93, 96,103,	32, 32, 34, 54,	5,7.5,	550
114	40.7	69, 74, 79, 79,	59, 61, 61, 64,	7.5,7.5,	400
115	40.7	71, 74, 91, 96,	47, 47, 49, 56,	7.5,7.5,	350
116	46.2	76, 78, 80,112,	37, 37, 39, 56,	6.5, 11,	500
117	46.2	82, 89, 91,102,	35, 37, 37, 43,	6.5,6.5,	500
118	46.2	67, 71, 87, 93,	35, 39, 41, 45,	6.5,6.5,	500
122	46.5	84, 95,101,114,	41, 43, 43, 45,	6.5,6.5,	400
124	46.5	86, 90, 95, 95,	67, 75, 80, 84,	37, 43,	50
126	46.5	101,108,114,116,	97,103,105,105,	90,101,	<10

Table 72e. continued.

Day 140. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

A.	D.	E. E. E. E.	F. F. F. F.	G. G.	H.
140a	40.6	84, 86, 89, 91,	44, 44, 44, 49,	7.5, 10,	400
142	40.7	74, 76, 76, 88,	44, 57, 59, 61,	5,7.5,	400
144	37.2	94, 97,108,121,	67, 67, 70, 75,	11, 14,	200
146	35.2	100,105,105,128,	85, 94,105,105,	77, 77,	10

Day 151. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

151a	34.8	81, 86, 89, 95,	43, 43, 49, 52,	8.5, 15,	400
154	34.6	75, 78, 84,104,	64, 69, 75,101,	11, 12,	200
157	30.9	107,113,130,139,	84, 97,130,142,	68, 71,	15

Day 161. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

161a	38.3	92, 92, 94,104,	52, 63, 63, 66,	5, 8,	500
164	41.3	83, 85, 87, 94,	78, 78, 80,100,	68, 70,	15
166	42.8	92, 94, 94, 96,	82, 89, 96,106,	68, 84,	15
168	43.6	71, 83, 83, 92,	87, 90, 92, 99,	69, 85,	15

Day 169. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

169a	46.6	84, 86, 88, 99,	30, 37, 41, 43,	6.5,6.5,	500,
173	74.8	86,107,126,131,	95, 99,104,119,	86,107,	<10

Day 176. Perfuser drained and refilled with 250 ml. of 1,000 ppm. 2,4-D solution. Perfuser re-started, sampled after 1 hr.

176a	62.2	76, 84, 93,100,	21, 27, 29, 42,	5,6.5,	750
180	48.9	108,111,113,115,	45, 61, 68, 76,	12, 12,	250
181	48.9	98,102,104,121,	100,110,129,135,	92, 98,	<10

Day 183. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Perfuser re-started, sampled after 2 hr.

A.	B.	C.	A.	B.	C.	A.	B.	C.
183a2hr	35.5	71,	184b	4.2	8,			

Day 184. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1/2hr.

184ba	104	208,	185	8.5	17,	186b	6	12,
184a½hr	74.5	149,						

Day 186. Perfuser drained and refilled with 250 ml. of 400 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

186ba	2x108	432,	187	2x39	156,	189	58	116,
186a1hr	2x 45	180,	188	2x35.5	142,	190	47	94,

Table 72e. continued.

A.	B.	C.	A.	B.	C.	A.	B.	C.
191	59	118,	194	53	106,	197	21	42,
192	56	112,	195	48.5	97,	198b	11.5	23;
193	56	112,	196	41	82,			

Day 198. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

198ba	95	190,	199b	25.2	50,			
198alhr	62	124,						

Day 199. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

199ba	85	170,	199alhr	60	120,	200b	14.2	28,

Day 200. Perfuser drained and refilled with 250 ml. of 300 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

200ba	2x78	312,	201	47	94,	202b	6.7	13,
200alhr	2x40	160,						

Day 202. Perfuser drained and refilled with 250 ml. of 400 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

202ba	2x91	364,	203	56	112,	205b	11	22,
202alhr	2x49	196,	204	40	80,			

Day 205. Perfuser drained and refilled with 250 ml. of 400 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

205ba	2x90	360,	206	54	108,	208b	7.0	14,
205alhr	2x56	224,	207	30.4	61,			

Day 208. Perfuser drained and refilled with 250 ml. of 400 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

208ba	2x97	388,	209	2x28.1	112,	211b	4	8,
208alhr	2x57	228,	210	27.5	55,			

Day 211. Perfuser drained and refilled with 250 ml. of 400 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

211ba	2x103	412,	212	61	122,	214b	14.7	29,
211alhr	2x56	224,	213	40	80,			

Day 214. Perfuser drained and refilled with 250 ml. of 400 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

214ba	2x108	432,	216	54.5	109,	218	24	48,
214alhr	2x60.5	242,	217	39.1	78,	219b	4.4	9,
215	75	150,						

Day 219. Perfuser drained and refilled with 250 ml. of 600 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

Table 72e. continued.

A.	B.	C.	A.	B.	C.	A.	B.	C.
219ba	4x74	592,	226	82	164,	234	55.5	111,
219alhr	4x46	368,	227	69	138,	235	57.7	115,
220	2x59	236,	228	78	156,	236	46.5	93,
221	92.5	185,	229	64	128,	237	36.5	73,
222	100	200,	230	66	132,	238	29.7	59,
223	93.5	187,	231	61.5	123,	239	10.6	21,
224	73	146,	232	56	112,	240b	6.3	13,
225	83	166,	233	61	122,			

Day 240. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

240ba	98	196,	241	20	40,	242b	3.6	7,
240alhr	55	110,						

Day 242. Perfuser drained and refilled with 250 ml. of 200 ppm. 2,4-DCP solution. Solution sampled before adding and after 1 hr.

242ba	110	220,	243	28	56,	244	3.1	6,
242alhr	42.5	85,						